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Cadmium Poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other Marine Organisms

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EISLER, R. 1971. Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. J. Fish. Res. Bd. Canada 28: 1225–1234.

In acute toxicity bioassays with $CdCl_2 \cdot 2\frac{1}{2}H_2O$ at 20 C and 20‰ salinity, the concentrations fatal to 50% of the organisms of various marine species in 96 hr ranged between 0.32 and 55.0 mg/liter Cd^{2+}. The order of sensitivity was: sand shrimp, *Crangon septemspinosa* (0.32); hermit crab, *Pagurus longicarpus* (0.32); grass shrimp, *Palaemonetes vulgaris* (0.42); common starfish, *Asterias forbesi* (0.82); common soft-shell clam, *Mya arenaria* (2.2); green crab, *Carcinus maenus* (4.1); Atlantic oyster drill, *Urosalpinx cinerea* (6.6); eastern mud snail, *Nassarius obsoletus* (10.5); sandworm, *Nereis virens* (11.0); striped killifish, *Fundulus majalis* (21.0); blue mussel, *Mytilus edulis* (25.0); sheepshead minnow, *Cyprinodon variegatus* (50.0); and mummichog, *Fundulus heteroclitus* (55.0). Mummichogs were more susceptible to cadmium exposures at 20 C than at 5 C and at 5‰ salinity than at 15, 25, or 35‰. Additional studies with mummichogs clearly demonstrated that 96 hr was not sufficient to adequately evaluate cadmium toxicity to this species. Mummichog whole body cadmium residues determined by atomic absorption provided a useful index of cadmium body burden among fish surviving exposure. However, cadmium residue data from dead mummichogs were of limited worth owing to accumulation after death.

EISLER, R. 1971. Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. J. Fish. Res. Bd. Canada 28: 1225–1234.

Des analyses biologiques d'empoisonnement aiguë par le $CdCl_2 \cdot 2\frac{1}{2}H_2O$ à 20 C et 20‰ S démontrent qu'après 96 heures, les concentrations fatales à 50% de diverses espèces halieutiques se situent entre 0.32 et 55.0 mg/liter de Cd^{2+}. L'ordre de sensibilité est le suivant: la crevette de sable, *Crangon septemspinosa* (0.32); le pagure, *Pagurus longicarpus* (0.32); le bouquet commun, *Palaemonetes vulgaris* (0.42); l'étoile de mer, *Asterias forbesi* (0.82); la mye commune, *Mya arenaria* (2.2); le crabe vert, *Carcinus maenas* (4.1); le perceur d'huitre, *Urosalpinx cinerea* (6.6); le naissaire des vases de l'Est, *Nassarius obsoletus* (10.5); l'arénicole des pêcheurs, *Nereis virens* (11.0); le barré, *Fundulus majalis* (21.0); la moule bleue, *Mytilus edulis* (25.0); le méné tête-de-mouton, *Cyprinodon variegatus* (50.0); et le choquemort, *Fun-*

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dulus heteroclitus (55.0). Les choquemorts sont plus sensibles à des expositions au cadmium à 20 C qu'à 5 C et à 5‰ S plus qu'à 15, 25 ou 35‰ S. Des expériences supplémentaires sur les choquemorts démontrent que 96 heures sont insuffisantes pour évaluer adéquatement les effets toxiques du cadmium sur cette espèce. Des résidus de cadmium du corps entier de choquemorts, mesurés par absorption atomique, nous ont fourni un indice utile de la charge corporelle de cadmium des poissons qui ont survécu à l'exposition. Toutefois, les données de résidu de cadmium provenant de choquemorts morts ont une valeur diminuée du fait qu'il y a accumulation après la mort.

Received April 5, 1971

THERE is no evidence that cadmium is biologically essential or beneficial; on the contrary, cadmium has been implicated as the cause of numerous human deaths (McKee and Wolf 1963; Kobayashi 1971) and, among aquatic species, various deleterious effects (Doudoroff and Katz 1953; Schweiger 1957; Harry and Aldrich 1958; Cabejszek and Stasiak 1960; Roberts 1963; Yager and Harry 1964, 1966; Pickering and Henderson 1966; Ishizaka et al. 1966; Mount and Stephan 1967; Ball 1967; Shuster and Pringle 1969; Gardner and Yevich 1969, 1970; Jackim et al. 1970). This, and the fact that seafoods are a major source of cadmium in the human diet and that cadmium salts are found in wastes discharged into the estuarine environment from electroplating plants, pigment works, lead mines, and other sources (McKee and Wolf 1963) has prompted the West Kingston laboratory to initiate a continuing series of studies on cadmium and its effects on marine organisms. The present account complements results of published studies conducted by investigators from the National Marine Water Quality Laboratory with the mummichog, *Fundulus heteroclitus*, an estuarine cyprinodontiform teleost, and cadmium chloride (Gardner and Yevich 1969, 1970; Jackim et al. 1970). Specifically, it reports on the acute toxicity of $CdCl_2 \cdot 2\frac{1}{2}H_2O$ to mummichogs and other marine species; and in mummichog, effects of salinity and temperature of the medium on cadmium toxicity, on posttreatment mortality after immersion in sublethal concentrations, and on cadmium residues during and following immersion in a range of Cd^{2+} concentrations.

General Methods

Reagent grade $CdCl_2 \cdot 2\frac{1}{2}H_2O$ in distilled water at 30 mg Cd^{2+} per milliliter constituted the cadmium stock for all studies. Aliquots of this stock were added to the assay containers about 60 min after the assay organisms to achieve the desired cadmium concentrations. There was less than 5% loss of cadmium from the aerated test medium (without organisms) during a period of 264 hr as determined by atomic absorption spectroscopy; this pattern remained unchanged over the entire range of Cd^{2+} concentrations used.

Bioassay procedures for evaluation of toxicity of cadmium to all test species were, with minor exceptions, identical to those of LaRoche et al. (1970). All assays were conducted under subdued natural light in aerated media containing dissolved oxygen concentrations from 4 to 6 mg/liter. Test containers were wide-mouth glass jars holding 3 liters of a synthetic seawater medium (Zaroogian et al. 1969) of *p*H 8.0 and, unless indicated otherwise, of 20‰ salinity and maintained at 20 C. In acute toxicity assays, dead organisms were removed and recorded every 24 hr. The TL_{100} values shown were the highest nonlethal concentrations tested; similarly, TL_0 values were the lowest concentrations tested producing 100% mortality. For each test species, TL_{25}, TL_{50}, and TL_{75} values were determined by graphical interpolation in plotting percent survival vs. log Cd^{2+} concentration (American Public Health Association 1971). Except where noted, all organisms used were actively feeding in the laboratory for at least 10 days prior to bioassay, with mortality for individual species <5% during the first 24 hr and <1% afterwards.

Whole body cadmium residues were determined for mummichog in several studies. This was accomplished by atomic absorption spectroscopy using methodology described in detail by Eisler and LaRoche (1971).

Selected Species: 96-hr Toxicity

This section reports on concentrations of cadmium fatal to selected species of marine crustaceans, molluscs, annelids, echinoderms, and teleosts.

METHODS

All species assayed (Table 1), except sandworm (*Nereis virens*), common starfish (*Asterias forbesi*), and Atlantic oyster drills (*Urosalpinx cinerea*), were collected during the spring months of 1969 and 1970 from the salt marsh at Jerusalem, Rhode Island. Sandworms were purchased from a local bait dealer who received his stock from a supplier in Maine; drills and starfish were collected from the intertidal zone of Narragansett Bay, in the area bordering the Graduate School of Oceanography of the University of Rhode Island. All animals were held for 14 days at 20 C and 20‰ salinity. This thermosaline regimen approximated that of the capture locales. Brine shrimp and chopped clams were fed to all groups except bivalves; blue mussels (*Mytilus edulis*) and the common soft-shell clams (*Mya arenaria*) were not fed. During the holding period and

TABLE 1. Mean lengths and weights of marine organisms, total numbers, and total weights per 3 liters of medium in study on 96-hr toxicity of cadmium.

Organism	Length (*mm*)	Wt (*g*)	No. animals	Wt/3 liters (*g*)
Crustaceans				
Hermit crab, *Pagurus longicarpus*	20[a, b]	0.47	108	1.41
Sand shrimp, *Crangon septemspinosa*	25[c]	0.25	60	0.75
Grass shrimp, *Palaemonetes vulgaris*	28[c]	0.33	108	1.00
Green crab, *Carcinus maenus*	37[c]	5.9	20	5.9
Molluscs				
Common soft-shell clam, *Mya arenaria*	48	5.2[d]	96	15.6
Blue mussel, *Mytilus edulis*	48	4.0[d]	96	12.0
Atlantic oyster drill, *Urosalpinx cinerea*	24[b]	0.60[d]	96	1.80
Eastern mud snail, *Nassarius obsoletus*	23[b]	0.56[d]	108	1.68
Echinoderms				
Common starfish, *Asterias forbesi*	52[e]	24.5	96	24.5
Annelids				
Sandworm, *Nereis virens*	195	8.0	96	24.0
Teleosts				
Striped killifish, *Fundulus majalis*	44	0.95	48	3.8
Sheepshead minnow, *Cyprinodon variegatus*	36	1.1	32	2.2
Mummichog, *Fundulus heteroclitus*	45	0.89	132	2.7

[a]Length of *Nassarius* shell in which crabs were housed.
[b]Apex to tip of siphonal canal.
[c]Eye to uropod.
[d]Meats only.
[e]Madreporite to tip of longest arm.

afterwards, mortalities in untreated groups were less than 1%. Animals were not fed for 48 hr prior to, or during, the 96-hr assay. The volume to weight ratios (Table 1) for green crab (*Carcinus maenus*), clam, mussel, starfish, and sandworm exceed the ratio recommended by American Public Health Association (1971) of 1 g per liter, and this may reduce the significance of the TL values obtained for these organisms.

After 14 days, organisms were distributed among the jars and each species tested separately. A minimum of eight test concentrations of Cd^{2+}, four replicates per concentration, and three animals per replicate was used for all species assayed except sand shrimp (*Crangon septemspinosa*), green crab, striped killifish (*Fundulus majalis*), and sheepshead minnow (*Cyprinodon variegatus*). For those four species it was necessary to use fewer concentrations and replicates owing to the small numbers of test organisms available (Table 1). Concentrations of Cd^{2+} tolerated by 25, 50, and 75% of each test species at 24, 48, and 96 hr were determined.

RESULTS

In general, the teleosts were most resistant with TL_{50} values for 96-hr exposures between 21 and 55 mg Cd^{2+}/liter, and the crustaceans most sensitive with values between 0.32 and 4.1 mg Cd^{2+}/liter (Table 2). Intermediate in toxicity were molluscs with corresponding values of 2.2, 6.6, 10.5, and 25.0 mg Cd^{2+}/liter and sandworm with a value of 11.0 mg Cd^{2+}/liter (Table 2). The marked and progressive decline in TL values between 24 and 96 hr indicated that some organisms from each of the species tested died during that interval.

There appeared to be a restricted range within which 50% of all mortality occurred during 96 hr. At this exposure the ratio TL_{25}/TL_{75} extended between 3.2 and 7.0 for crustaceans, 2.1 and 2.6 for molluscs (exception was mussel with TL_{25} (96 hr)/TL_{75} (96 hr) = 24.2), 2.1 and 7.8 for teleosts, 4.8 for starfish, and 3.2 for sandworm (Table 2).

Mummichog: Effects of Temperature and Salinity

The influence of two temperatures and four salinities (8 thermosaline regimens) on cadmium-induced mortality of mummichog (*Fundulus heteroclitus*) are presented.

METHODS

A total of 480 mummichogs of mean length 53 mm from a single collection were maintained in holding aquaria at 20 C and 20‰ salinity for 10 days. Four

TABLE 2. Concentrations of cadmium, as $CdCl_2 \cdot 2\frac{1}{2}H_2O$, allowing survival of 75, 50, and 25% of organisms of selected marine species at 20 C and 20‰ salinity at three time intervals. Values are in mg/liter Cd^{2+} added to medium at start.

Species	24 hr			48 hr			96 hr		
	TL_{75}	TL_{50}	TL_{25}	TL_{75}	TL_{50}	TL_{25}	TL_{75}	TL_{50}	TL_{25}
Sand shrimp	1.4	2.4	4.1	0.29	0.50	0.85	0.08	0.32	0.56
Hermit crab	4.1	>200	>200	2.3	3.7	10.0	0.18	0.32	0.57
Grass shrimp	17	43	50	2.8	5.8	10.0	0.18	0.42	1.00
Common starfish	4.3	12	19	0.54	1.00	15.5	0.50	0.82	2.40
Common soft-shell clam	>200	>200	>200	40	50	160	1.5	2.2	3.2
Green crab	40	100	>100	8.2	16.6	100	2.0	4.1	8.2
Atlantic oyster drill	125	158	195	21	28	32	4.4	6.6	10.5
Eastern mud snail	>200	>200	>200	88	125	200	6.4	10.5	16.6
Sandworm	19	25	31	19	25	31	5.6	11.0	18.0
Striped killifish	100	125	160	4.1	59	84	4.1	21.0	32.0
Blue mussel	>200	>200	>200	100	165	200	7	25	170
Sheepshead minnow	70	100	140	29	50	72	10	50	72
Mummichog	>100	>100	>100	>100	>100	>100	35	55	74

groups of 60 fish were then held for 14 days in aquaria of 70 liters capacity at a salinity of 5, 15, 25, or 35‰; aquaria were in a temperature-controlled room maintained at 5 ± 1 C. Four similar groups were held at 20 ± 1 C and the same four salinities. The medium used in all instances was the synthetic formulation described by Zaroogian et al. (1969). Salinities were adjusted to the desired level by varying the amount of distilled water added. Mummichogs fed actively on brine shrimp during the holding period. After 14 days, each group was bioassayed for 192 hr in newly prepared media of identical temperature and salinity using procedures listed in the general methods section. Each of the eight assays consisted of five Cd^{2+} concentrations, four replicates per concentration, and three fish per replicate.

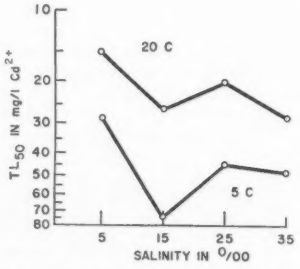

FIG. 1. Concentrations of cadmium allowing 50% survival of mummichogs at 192 hr during exposures at two temperatures and four salinities. Each point represents 60 fish.

RESULTS

Mummichogs were more susceptible to cadmium at 20 C than at 5, by factors that ranged between 1.7 and 3.0 (Fig. 1). Mummichogs were most susceptible at 5‰, the lowest salinity tested, at both temperatures. The effect of the higher salinities on toxicity was not readily apparent; however, within each temperature regimen, fish held at 15‰ were, in general, more resistant than those at higher and lower salinities.

Mummichog: Cadmium Residues After 264 hr

This portion reports on concentrations of cadmium lethal to mummichog at 20 C and 20‰ salinity during a 264-hr period, and on whole body cadmium content of fish that survived exposure for this interval or died during exposure.

METHODS

One hundred and thirty-two mummichogs averaging 55 mm in length and 1.1 g in weight from a single seine collection were bioassayed against 11 concentrations of Cd^{2+}, namely, 0 mg/liter (controls), 0.1, 0.4, 1, 4, 10, 40, 75, 100, 200, and 400 mg/liter. There were four replicates per concentration, and three fish per replicate. All fish that died during the 264-hr assay, and were removed during the daily inspection, were rinsed in distilled water and frozen. Fish that survived immersion for 264 hr were narcotized in M.S. 222 prepared in distilled water (200 mg/liter), then frozen. Cadmium residues of pooled fish receiving the same treatment were determined by atomic absorption.

Details of the dry-ashing procedure and sample preparation are described by Eisler and LaRoche (1971).

Results

Concentrations of cadmium tolerated by mummichogs at 24, 96, 168, and 264 hr are listed in Table 3. TL_{25}, TL_{50}, and TL_{75} values at 96 hr were in good agreement with comparable data (Table 2). TL_{100} and TL_0 values remained unchanged between 96 and 264 hr; however, there was substantial mortality at intermediate concentrations during the extended immersion period. As a result, TL_{50} values at 168 and 264 hr were 82 and 46% respectively, of that at 96 hr.

Among survivors, highest Cd residues were in fish from the 4 and 10 mg Cd^{2+}/liter groups; values were 137 and 113 mg/kg ash, respectively (Fig. 2). Cadmium residues among fish that died varied directly with Cd^{2+} in the medium at the start; values ranged from 156 mg Cd/kg whole body ash for the 40 mg Cd^{2+}/liter group to 6440 mg Cd/kg body ash for the 400 mg Cd^{2+}/liter group. Cadmium content in controls as well as those surviving immersion for 264 hr in 0.1 mg Cd^{2+}/liter was less than 7 mg Cd/kg whole body ash (Fig. 2) (the sensitivity limit of the analytical procedure). Fish subjected to 0.4 mg Cd^{2+}/liter and higher exhibited measurable concentrations of cadmium in whole body ash (Fig. 2).

On the basis of whole body cadmium content it appears that patterns of concentration were different for survivors and for fish that died. Survivors, in general, contained progressively smaller percentages of the total cadmium available in the medium with increasing Cd^{2+} levels (Fig. 2), whereas fish that died contained progressively larger percentages. The percent uptake value for the 40 mg Cd^{2+}/liter group of dead mummichog is low; two fish from this lot survived but were not included with the dead fish for analysis by atomic absorption.

Table 3. Concentrations, in mg/liter Cd^{2+} added to medium at start, that allowed survival of various percentages of mummichog at 20 C and 20‰ salinity.

	Hours			
	24	96	168	264
TL_{100}	75	10	10	10
TL_{75}	115	40	20	15
TL_{50}	140	49	40	23
TL_{25}	175	61	55	35
TL_0	400	75	75	75

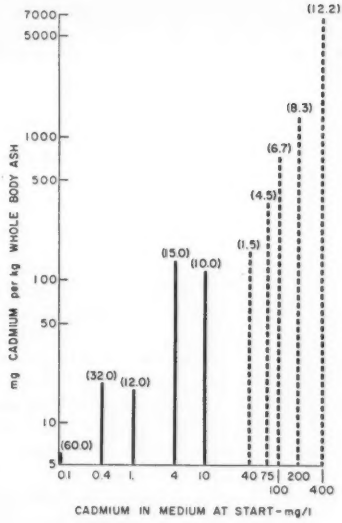

Fig. 2. Cadmium residues in mummichogs surviving immersion in various Cd^{2+} concentrations for 264 hr (solid bars), or dying during immersion (broken bars). Values in parentheses are percentages of cadmium in medium removed by fish.

Mummichog: Posttreatment Effects

Effects observed in this experiment were: (*a*) mortality of fish during an observation period of approximately 7 weeks after short-term immersion in various concentrations of Cd^{2+}; and (*b*) whole body cadmium residues of survivors immediately after exposure and at about 7 weeks later.

Methods

Groups of fish were subjected to Cd^{2+} concentrations of 10, 40, or 75 mg/liter for 6, 24, 48, and 96 hr at 20‰ salinity and 14–16 C. Each group consisted of 24 mummichogs of mean length 50 mm from a common collection. Fourteen such groups were employed, i.e., 3 concentrations × 4 exposure periods + 2 control groups, or a total of 336 mummichogs.

Immediately after exposure, 12 survivors from each group were transferred to individual 20-liter glass jars containing 19 liters of aerated cadmium-free (<0.03 mg/liter) media at 20‰ salinity. The remaining live fish were frozen and retained for cadmium residue determinations. During the posttreatment observation period survivors were fed an excess of thawed adult brine shrimp once daily. Dead fish were removed every 24 hr, recorded, and discarded. About 75% of the medium was renewed every 10 days. The study was terminated when no deaths were observed in any group for 14 consecutive days.

Whole body cadmium content in a single pooled sample of live mummichogs from each of the 14 test groups was determined at two time intervals: immediately after cadmium exposure, and 50 days after the start of the study, i.e., 46.0–49.75 days after exposure. Results were analyzed using statistical three-way analysis of variance techniques to test for differences between exposure periods, concentrations, and time intervals (Chapman MS 1955).

Results

The large numbers of deaths in the posttreatment intervals (Fig. 3) show that cadmium-induced toxicity in mummichogs cannot be adequately evaluated during an exposure period of 96 hr. The posttreatment mortality appeared to be a direct function of initial concentration and of exposure period; highest mortality was observed in groups subjected to initial concentrations of 40 and 75 mg Cd^{2+}/liter for intervals of 24 hr or longer. Fish that died during the posttreatment period were moribund and refused to feed for several days prior to death.

Groups that exhibited less than 10% mortality during the posttreatment period (Fig. 3) contained 85.3 mg Cd/kg whole body ash and lower immediately after immersion in Cd^{2+} solutions (Table 4); groups showing substantial posttreatment mortality had cadmium levels of 92.6 mg/kg whole body ash and higher. Fish that were immersed in 75 mg/liter Cd^{2+} for 48 and 96 hr contained, just after exposure, lower body residues than the group exposed for only 24 hr. As mummichogs subjected to 75 mg/liter Cd^{2+} for 48 and 96 hr experienced some deaths during treatment, and were removed, the residues in the dead fish may have been sufficient to decrease the cadmium available for survivors.

Cadmium losses during the posttreatment period were 54% and 76% for groups exhibiting no appreciable mortality during the observation period;

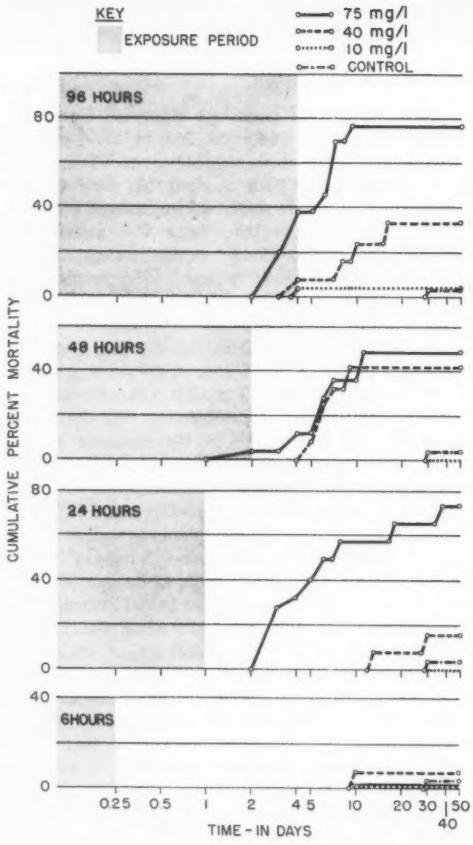

FIG. 3. Mortalities of mummichogs during and after immersion for four periods (6–96 hr) in three concentrations of cadmium (mg/liter) at 20 C and 20‰ salinity.

TABLE 4. Concentrations of cadmium, in mg of Cd/kg whole body ash, in mummichogs at 0 and 50 days after surviving exposure to three concentrations of Cd^{2+} for four periods at 14–16 C and 20‰ salinity.

Initial concentration of Cd^{2+} (*mg/liter*)	Exposure period (*hr*)							
	6		24		48		96	
	0 days	50	0	50	0	50	0	50
0	–	–	–	–	–	–	<7.	<7.
10	20.6	8.1	24.4	<7.	25.1	10.1	39.8	13.3
40	60.1	27.6	92.6	22.3	119.3	49.7	134.6	45.5
75	85.3	28.9	386.8	68.8	287.5	76.2	195.1	83.2

values were essentially the same (57–82%) for groups with high death rates (Table 4).

Results of statistical variance analysis of data from Table 4 suggest that whole body cadmium is lower at 50 days after treatment than immediately after immersion ($F_{(1,17\ df)} = 18.3$; significant at the 0.01 level), and that mummichog subjected to higher initial Cd^{2+} concentrations contained higher body residues ($F_{(2,17\ df)} = 5.6$; significant at the 0.05 level). Duration of exposure between 6 and 96 hr did not affect whole body residues ($F_{(3,17\ df)} = 1.2$; not significant at the 0.05 level). The latter result is attributed primarily to the comparatively low residue content of survivors measured immediately after exposure to 40 and 75 mg/liter Cd^{2+} for 96 hr (Table 4).

Cadmium Accumulation and Retention by Dead Mummichog

This study was conducted to determine if dead fish can accumulate cadmium from the medium over a 48-hr period at the same rate as living fish and retain these levels after transfer to uncontaminated media for 24 hr.

Methods

A total of 180 mummichogs from a single collection, of mean length 50 mm, were subdivided into 5 lots of 36. One lot of live fish, consisting of 12 replicates of 3, was immersed for 24 hr at 20 C and 20‰ salinity in a synthetic seawater formulation containing 40 mg/liter Cd^{2+}, a high sublethal concentration. At 24 hr, half the fish were narcotized in uncontaminated media containing 200 mg/liter M.S. 222, rinsed in distilled water for 60 sec, and subsequently analyzed for whole body cadmium. The remaining live fish were transferred to cadmium-free (<0.03 mg/liter Cd^{2+}) media for 24 hr of identical temperature and salinity, then prepared as above for cadmium analysis. A second group of live mummichogs was treated in an identical manner except that initial exposure was for 48 hr instead of 24 hr. Concomitantly, the experiment was repeated with dead mummichogs, quick-frozen at 0 C and thawed at room temperature (groups 3 and 4). Living ($n = 18$) and dead ($n = 18$) controls were held in uncontaminated media for 72 hr and then analyzed for whole body cadmium.

Results

Dead mummichogs accumulated much more cadmium than living animals: 53 times as much in the 24-hr immersion and 89 times as much after immersion for 48 hr (Table 5). The residues in the fish that survived the 24- and 48-hr exposures were about 0.04 and 0.05%, respectively, of the total cadmium in the medium at the beginning of the test, in comparison with 2.2 and 4.3% for the dead fish.

Average loss of whole body cadmium at 24 hr after treatment in fish that survived exposure for 24 and 48 hr was 40.6 and 12.0%, respectively, in comparison with 48.9 and 36.2% for dead mummichogs.

Discussion

The acute toxicity values in the present study indicated that teleosts were more resistant to cadmium (TL_{50} for 96-hr exposures = 21–55 mg/liter Cd^{2+}) than all marine invertebrates studied except mussel (TL_{50} for 96-hr exposures = 0.32–11.0 mg/liter Cd^{2+}). There is ample evidence from this study and from others (Doudoroff and Katz 1953; Schweiger 1957; Ball 1967; Shuster and Pringle 1969) that 96 hr is not of sufficient duration to evaluate the toxicity of cadmium to aquatic species. For example, oysters that were continuously subjected to flow-through concentrations of 0.2 mg/liter of Cd^{2+} began to die only after the 4th week of exposure and all died by the 17th week (Shuster and Pringle 1969). However, the question whether the 96-hr test period is adequate to assess the relative sensitivity of species to cadmium is yet to be resolved.

Mummichogs rank among the more resistant species to cadmium. In studies of 264 hr duration, the concentration of cadmium at which survival time nearly equalled that of controls was 10 mg/liter Cd^{2+} for mummichog (Table 3), but was between 0.03 and 0.2 mg/liter Cd^{2+} for various species of freshwater fishes and invertebrates (Doudoroff and Katz 1953; Schweiger 1957). Pickering and Henderson (1966) reported TL_{50} (96-hr) values with four species of freshwater teleosts from 0.63 to 2.8 mg/liter Cd^{2+} in water of 20 mg/liter hardness, and from 66 to 73 mg/liter Cd^{2+} in water of 360

Table 5. Concentrations of cadmium, in mg Cd/kg whole body ash, in dead and living mummichogs at 0 and 24 hr after exposures of 24 and 48 hr to cadmium at an initial concentration of 40 mg/liter Cd^{2+}.[a]

Hr after exposure	24 hr		48 hr	
	Living	Dead	Living	Dead
0	56.8	3040	67.6	6014
24	33.8	1554	59.5	3835

[a]Values from live and dead controls after 72 hr immersion were less than 7 mg Cd/kg ash.

mg/liter hardness. Average survival time of juvenile eels, *Anguilla japonica*, subjected to 1.1 mg/liter Cd^{2+} was 18 hr at 20 C (Oshima, cited in Doudoroff and Katz 1953).

It is concluded that concentrations as low as 0.1 mg/liter Cd^{2+} in the medium, or whole body burdens in excess of 86 mg Cd/kg ash, are potentially hazardous to mummichogs and probably other marine species. Fish that contained more than 86 mg Cd/kg sustained heavy mortality during an observation period of 50 days in cadmium-free sea water. Exposure for 264 hr to concentrations as low as 4 mg/liter Cd^{2+} can also produce body Cd levels in excess of 86 mg/kg ash (Fig. 2). This does not imply that whole body levels of Cd lower than 86 mg/kg ash were harmless to mummichog. Evidence exists (Eisler and LaRoche 1971) that cadmium residues in whole mummichog collected from waters of Rhode Island between 1967 and 1970, were always less than 7 mg Cd/kg whole body ash regardless of age, size, or sex of this species, season of collection, and temperature or salinity of the medium. On this basis, only a single exposure concentration tested, 0.1 mg/liter Cd^{2+} for 264 hr, produced cadmium residues not grossly different from controls.

0.1 mg/liter Cd^{2+} represented 1/550th the TL_{50} (96 hr) value for Cd^{2+} and mummichog. If the same application factor of 1/550 were applied to cadmium toxicity data listed herein for sand shrimp or hermit crab, then cadmium concentrations in excess of 0.00058 mg/liter could be considered potentially hazardous to these species. It is not known at this time if the application factor of 1/550 is unrealistically stringent for sand shrimp and hermit crab. Nevertheless, the derived value of 0.00058 mg/liter Cd^{2+} exceeds by a factor of 5 the accepted representative value of 0.00011 mg/liter cadmium, range = 0.00006–0.00017 mg/liter, in offshore marine waters (Johnston 1965). Incidentally, the 1962 Drinking Water Standards of the U.S. Public Health Service set a mandatory limit of 0.01 mg/liter for cadmium (McKee and Wolf 1963).

Rats and dogs are relatively resistant to cadmium when compared to aquatic species. There were no histopathological effects or measurable change in body weight, growth rate, food or water consumption, and hemoglobin content of rats (Decker et al. 1956a, b) and dogs (Decker et al. 1956b; McKenzie et al. 1957) that imbibed up to 10 mg/liter of Cd^{2+} in drinking water for periods up to 1 year. Humans, unlike laboratory animals, experienced "itai-itai," a painful and crippling disease caused by chronic poisoning of heavy metals (Kobayashi 1971). The disease was especially prevalent among older women residing in certain areas of Japan. Bones of afflicted individuals exhibited extreme demineralization, and were prone to fracture under slight strain. Autopsies indicated abnormally high residues of zinc, lead, and especially cadmium in bone and internal organs. Kobayashi (1971) concluded that "itai-itai" disease was caused by life-long ingestion of water and crops contaminated by cadmium.

Bioconcentration of radiocadmium by several species of edible marine fishes, shellfish, and algae decreased with augmented levels of stable cadmium in the medium (Hiyama 1962). Similar results were observed in the present study with mummichogs surviving immersion in various concentrations of Cd^{2+}. The pattern of uptake by mummichogs that died during immersion in high levels of Cd^{2+} was contrary to that exhibited by living animals. Subsequent studies disclosed that data on cadmium residues in dead mummichogs should be interpreted with extreme caution. For example, cadmium residues in fish dead prior to immersion in Cd^{2+} solutions were, after exposure, up to 89× higher than levels accumulated by living fish (Table 5).

Biochemical and histopathological aspects of acute cadmium poisoning in mummichog have been reported. Jackim et al. (1970) showed that alkaline phosphatase was the most sensitive liver enzyme studied in vitro, with 27% inhibition at 0.12 mg/liter Cd^{2+}. In vivo studies of 96-hr duration at 27 mg/liter Cd^{2+} indicated that alkaline phosphatase activity in liver was not significantly different from controls; however, inhibition was observed for acid phosphatase, xanthine oxidase, RNAase and catalase (Jackim et al. 1970). Gardner and Yevich (1970) observed histopathology of mummichog in the intestinal tract after 1 hr, in the intestinal mucosa after 2 hr, in the kidney after 12 hr, and in the gills after 20 hr, during exposure to 50 mg/liter Cd^{2+}. Incidence of lesions in mummichog exposed to 50 mg/liter Cd^{2+} is affected by *p*H, temperature, and salinity of the medium (Gardner and Yevich 1969). More lesions were observed in moribund fish at *p*H 4 than at *p*H 7.8, at 30 than at 20 C, and at 15 than at 30‰ salinity. The present study showed that mummichog were most sensitive to Cd^{2+} at comparatively high temperatures and at low salinities, which is in good agreement with the findings of Gardner and Yevich (1969).

It was resolved from the present investigation that mummichog acclimated to various salinities for 14 days were more sensitive to cadmium at low than high salinities. Mummichogs possess a highly efficient system for long-term regulation of the internal environment in external media of widely

different salinities (Pickford et al. 1969). However, mummichogs that were transferred to lower salinities, when compared to other species of euryhaline marine teleosts, were inhibited in their ability to effect an immediate reduction in haemal ionic outflux (Pickford et al. 1969). It was possible that internal stress produced as a result of inefficient physiological hemodilution processes in media of decreased salt content may have contributed to the observed susceptibility of mummichogs to cadmium at 5‰ salinity.

The exact mechanism of acute cadmium poisoning is unknown. Among teleosts, there is evidence linking cadmium poisoning with disrupted respiratory processes (Schweiger 1957; Mount and Stephan 1967; R. Eisler and J. C. Mallet unpublished data; Gardner and Yevich 1970), with kidney damage (R. Eisler and J. C. Mallet unpublished data; Gardner and Yevich 1970), and with whole body cadmium burdens (this paper). Schweiger (1957) reports that freshwater fish and invertebrate species subjected to high sublethal concentrations of cadmium for 7 days experienced difficulty in respiring and died after treatment. Schweiger concluded that the toxic action of cadmium was due to suffocation caused by deposition of dead cellular material on the gills and body surface. Others have found that cadmium concentrated in gill tissue (Mount and Stephan 1967) and caused damage to the gills (Gardner and Yevich 1970). Pathology and high Cd residues were also observed in the gills of adult tautog, *Tautoga onitis* (Pisces, Labridae) that died after immersion in 400 mg/liter Cd^{2+} at 20‰ salinity and 20 C for 8 hr (R. Eisler and J. C. Mallett, unpublished data). Prior to death, tautog heart rate had declined to 85% of normal and this was interpreted as due to the destruction of the respiratory function of the gill. There were no histopathological effects in gills, kidney, or intestine of tautog surviving exposure to 400 mg/liter Cd^{2+} for 4 hr (G. R. Gardner personal communication); however, these fish had a lower heart beat rate, very high levels of cadmium in gills, with about half this amount in kidneys. The effects of lower concentrations of cadmium and longer exposure periods on tautog heart beat rate, histopathology, and tissue residues were also investigated (R. Eisler and J. C. Mallett unpublished data). Tautog that survived immersion in 40 mg/liter 1 Cd^{2+} for 8 days exhibited no measurable change in heart beat rate. Cadmium residues were about six times higher in kidney than in gill and this represented a reversal from tautog subjected to 400 mg/liter Cd^{2+} for 4 hr. Tautog exposed for 8 days exhibited necrosis of the respiratory epithelium, sloughing of the intestinal mucosa, and destruction of the proximal convoluted tubules of the kidney (G. R. Gardner personal communication). R. Eisler and J. C. Mallett (unpublished data) concluded that the concentration of cadmium in the medium and the duration of exposure will affect responses of adult tautog, viz. under conditions of high concentration and short exposure the gill appears to be the primary site of damage, but under conditions of prolonged exposure and comparatively low Cd^{2+} levels the intestine, kidney, and possibly other tissues are more measurably affected.

From a practical viewpoint, the present investigation shows that levels as low as 0.1 mg/liter Cd^{2+} in the medium is potentially harmful to mummichogs and probably other marine species, that mummichogs with whole body residues in excess of 86 mg Cd/kg body ash will probably die within 5 weeks, and that cadmium residue data from mummichogs already dead on collection is of limited worth owing to possible accumulation after death. Preliminary studies that I recently (June 1971) terminated with mummichogs suggests that cadmium uptake and toxicity is affected markedly by salts of other heavy metals in solution, especially Zn and Cu, and that radiocadmium (Cd^{115m}) selectively concentrates in viscera. It is possible that future studies with selected tissues may provide useful information for a valid autopsy technique in aquatic organisms.

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An Experiment with Medaka, *Oryzias latipes*, and a Critique of the Hypothesis that Teleost Egg Size Controls Vertebral Count

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In each of two sets of experiments, two female medaka, *Oryzias latipes*, that laid different-size eggs were crossed reciprocally with two males. Vertebral counts of the offspring differed significantly between batches, but there was no consistent correlation between egg size and vertebral count. Both the highest and lowest counts resulted from crosses with females laying large eggs. Other experiments are reviewed, and it is concluded that within the same race of fish there is no causal connection between vertebral number and egg size, although between different races or related species large adult size is often correlated with high vertebral count. The possible advantages of variation in vertebral number are discussed.

LINDSEY, C. C., AND M. Y. ALI. 1971. An experiment with medaka, *Oryzias latipes*, and a critique of the hypothesis that teleost egg size controls vertebral count. J. Fish. Res. Bd. Canada 28: 1235–1240.

Au cours de deux séries d'expériences, deux medaka femelles, *Oryzias latipes*, pondant des oeufs de grosseurs différentes, furent croisées réciproquement à deux mâles. Le compte vertébral des descendants différa significativement, mais il n'y eu pas de corrélation constante entre la grosseur de l'oeuf et le nombre de vertèbres. Les comptes vertébraux le plus élevé et le plus bas ont tous deux été le produit de croisements de femelles à gros oeufs. Nous passons en revue d'autres expériences et en arrivons à la conclusion qu'au sein d'une même race de poissons il n'y a pas de relation causale entre le compte vertébral et la grosseur de l'oeuf. Par ailleurs, entre races différentes ou espèces apparentées, une grande taille à l'état adulte est souvent associée à un nombre vertébral élevé. Nous examinons les avantages possibles de la variation du nombre de vertèbres.

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MERISTIC variation in fishes has been the subject of quite a few experimental studies (recently reviewed by Fowler 1970) and of innumerable taxonomic investigations. Almost no satisfying hypotheses have resulted concerning either the evolutionary significance of meristic variation or the embryonic mechanisms that produce it. Latitudinal clines in the numbers of vertebrae, fin rays, or other meristic elements are widespread in nature, but their explanation is unknown. Meristic variation amongst the individuals of a wild fish population is often spectacular, yet its selective value remains inscrutible. Many different meristic series in fish can be altered by many different environmental factors, but few generalizations can be found either to predict these responses, or to ascribe them to any developmental laws.

There has been one hypothesis advanced that deserves attention but has not been adequately tested. This suggests that the size of the egg (or the amount of yolk) exerts an influence on the number of vertebrae in the larva. In 1926 Kyle wrote "··· it is the general rule that the smaller eggs yield the smaller larva and the smaller larva has the smaller number of vertebrae." Kyle offered no experimental evidence.

In 1959, Garside and Fry capitalized on a "natural experiment" by counting the myomeres on partly twinned salmonid embryos. They found that myomere counts were progressively lower in embryos that had a progressively more extensive

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degree of twinning; this correlation was significant in one species, *Salvelinus fontinalis*, but not in another that had a larger egg, *S. namaycush*. They concluded that there may perhaps be a certain critical yolk size, below which yolk size becomes limiting on the number of vertebrae formed, but above which (as in the *S. namaycush* eggs) yolk supply was ample for even complete twinned embryos each to form a full vertebral complement.

To test the hypothesis that egg size affects vertebral count, we have used a species of cyprinodont fish, *Oryzias latipes*, the medaka, which lays very much smaller eggs than do salmonids. Females have been chosen that produced eggs of significantly different sizes, and the vertebrae have been counted in offspring arising from these eggs when fertilized with different males. The results are compared, in the Discussion, with other studies that may be relevant to the relation of egg size to vertebral count in fishes.

Materials and Methods

Eggs were obtained from the same domestic stock of *O. latipes* (Timminck and Schlegel) that was used for experiments described by Lindsey and Ali (1965). Methods of obtaining eggs, rearing fish in cloth baskets suspended in a water bath held constant at 24 C, and counting vertebrae were as previously described. Two pairs of parents, females A and B, males E and F, were used in one set of reciprocal crosses, and two other pairs, females C and D, and males G and H, were used in a second set. For each set, two females had been selected that had different egg sizes (Table 1). Diet and living conditions were the same for all parents. Each individual egg was measured before it had completed first cleavage, using a binocular microscope and ocular micrometer. The diameter of the yolk (clearly visible through the transparent chorion) was recorded by averaging two measurements taken from different viewing positions. Neither measured diameter passed through the animal pole.

Each of the eight egg batches (Table 1) was the accumulation of the eggs laid by one female on two or more mornings. Previous experiment had shown that no significant vertebral differences occurred between young from two egg batches laid by the same mother on 2 days separated by as much as a 15-day interval (Ali MS 1962). Numbers of fertilized eggs per batch ranged from 98 to 164. Percentage of eggs that hatched ranged from 49 to 72%, and the percentage that survived to preservation from 34 to 59%. Time to 50% hatching ranged from 263 to 524 hr. Neither hatching time nor survival rate seemed to be correlated with egg size or vertebral count.

Results

Mean egg (i.e., yolk) diameters (shown in Table 1) differed significantly ($P < .01$) between females A and B, and also between females C and D. Mean vertebral counts differed greatly between different batches. The difference between the highest and lowest means, 1.51 vertebrae, is greater than the largest difference induced upon any single genotype within this stock of medakas by manipulation of temperature or other environmental factors (Ali MS 1962; Lindsey and Ali 1965).

Although egg diameters and vertebral counts each differed significantly between batches, no correlation was apparent between the two (Fig. 1). Two males (G and H) produced more vertebrae when crossed with *large* than with small eggs (both differences significant), but the other two males produced more vertebrae when crossed with *small* than with large eggs (one difference significant). Both the highest and lowest vertebral counts resulted from crosses (DH and BF) with

TABLE 1. Egg sizes and vertebral counts in two sets of reciprocal crosses.

Parents		Egg diam	Vertebrae				
♀	♂	(*mm*)	29	30	31	32	Mean
Exp 1							
A	E	1.03		4	30	4	31.00
A	F	1.06	1	23	12		30.31
B	E	1.13	1	19	15	1	30.44
B	F	1.14	3	28	5		30.05
Exp 2							
C	G	1.09		18	22		30.53
C	H	1.09		6	47	1	30.91
D	G	1.16		1	27	14	31.31
D	H	1.14			17	23	31.56

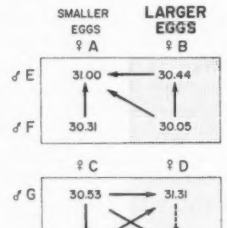

FIG. 1. Mean vertebral counts in two sets of reciprocal crosses. Arrows point towards significantly higher of two connected counts; solid arrows $P < .01$, broken arrow $P < .02$.

large eggs. Therefore, although females exerted some influence on vertebral count, the operative mechanism was evidently not yolk size. In all four tests two different males crossed with the same female produced significantly different counts (Fig. 1).

In three other meristic series (pectoral, dorsal, and total caudal ray counts) there was also no correlation between egg size and meristic count. No consistent parental influence was found in dorsal or total (i.e., major plus minor) caudal rays, but pectoral rays seemed to be more influenced by the father than by the mother. Only anal fin ray counts were correlated with egg size; the larger eggs in all four pairs of crosses yielded more anal rays than did the smaller eggs mated with the same male. Anal fin ray counts, however, were found (Ali MS 1962) sometimes to vary between successive daily egg batches from the same parent, and to be altered by mechanical shock and certain other external influences (none of which affected vertebral or pectoral ray counts), so the apparent correlation between anal ray count and egg size is of dubious significance.

Discussion

If a causal connection were demonstrated between the number of vertebrae in a fish and the size of the egg that produced it, there might be far-reaching implications. In many species the eggs laid by small females are smaller than those laid by large females (in *Clupea harengus harengus* (Hempel and Blaxter, 1967); in *Engraulis anchoita* (Ciechomski 1966); in *Salmo gairdneri* (Pennask Lake population, Scott 1962); in *Oncorhynchus nerka* (Bilton and Jenkinson MS 1966); and in *Platichthys flesus* (Solemdal 1967)). Eggs laid early in the season are commonly larger than those laid later (Hiemstra 1962). Hence, offspring of genetically similar fish, or of the same female in successive years, might have different mean vertebral counts even if reared under uniform conditions. One consequence would be to complicate the use by biologists of meristic characters in identifying discrete spawning stocks. Another would be the possibility that selection with respect to vertebrae might operate differentially on the offspring of young and of old spawners.

Salinity of the medium surrounding the female can alter the volume of the eggs that she produces (Solemdal 1967), although this would not necessarily alter the vertebral count of her offspring if count were dependent on dry weight rather than on overall volume of the egg. Bagenal (1969) suggests that egg size should always be measured as dry weight or chemical composition, since wet weight may be only a reflection of the time of spawning. In any event, *both* wet and dry egg weights were probably different in the experimental studies of egg size and vertebral count reported here, since neither salinity nor laying time differed consistently in the production of the large and small eggs studied.

If larger eggs produce fry that are longer and that have more vertebrae (Kyle 1926), then a correlation might be expected between the lengths of recently hatched fry and their vertebral counts. In wild fish such a correlation is frequently observed, but in many species it is probably attributable to temperature changes during a protracted spawning time. Templeman and Pitt (1961) cite several examples of species in which the larger fish within a year-class have more vertebrae, and these are all species that spawn on a rising temperature; conversely, in races of *C. harengus harengus* that spawn in autumn on a declining temperature, the larger fry have *fewer* vertebrae. However, these sorts of correlations are not necessarily due to direct modification of the embryo by temperature, since capacity for growth might be genetically linked to vertebral count. Amongst wild populations there are too many complexities for us to establish causal connection between egg size and vertebral count.

However, if egg size controls vertebral count, then amongst laboratory-reared fish that have been hatched from common parents and reared at uniform temperature we should expect to find a correlation between fry size and vertebral number. In fact *no* such correlation has been found in most of the experiments where it has been sought. Mottley (1937) showed that in one brood of *S. gairdneri* the longer fry had significantly more vertebrae, but since these fish were 5 months old before they were measured, their size hierarchy did not necessarily reflect the size hierarchy of the eggs from which they had hatched, and Mottley did not report whether a size–vertebral count correlation existed in the other 19 samples of *Salmo* that he raised. Dannevig (1950) found a correlation between vertebral count and length of fry from several females of *Pleuronectes platessa* that had spawned in an aquarium, but when he repeated the experiment the next year using a single female he found no such correlation. Other workers who have tested for this correlation in experimentally reared fish have found none: in *C. harengus harengus* (Hempel and Blaxter 1961), *S. gairdneri* (Canagaratnam MS 1959; Orska 1962 p. 331), *Salmo trutta* (Tåning 1952 p. 188), *O. nerka* (Canagaratnam MS 1959; Lindsey 1958), *Oncorhynchus gorbuscha*

(Canagaratnam MS 1959), *Catostomus commersoni* (Rough MS 1961), *O. latipes* (Ali MS 1962), and *Gasterosteus aculeatus* (Lindsey 1962).

In seeking a correlation between egg size and vertebral count, direct measurement of egg size differences would be preferable to inferring them from differences in fry length, but this has seldom been attempted. From the experiments on *O. latipes* reported here, vertebral count in this species is evidently controlled as much by the male as by the female, and some females with large eggs tend to produce fry with low counts. It has therefore been established that control of vertebral number has at least a large genetic component independent of egg size; it has not, however, been established that egg size has *no* effect, since large and small eggs with the same genotype were not compared.

In an earlier experiment on *S. gairdneri* taken from the outlet of Cultus Lake, B.C., in 1959, (referred to in Lindsey 1962 p. 307) the eggs from a single anadromous female were fertilized and sorted mechanically into small, medium, and large sizes by rolling them down an inclined trough whose bottom contained a tapering slot. The three lots of eggs were hatched and reared separately under identical conditions. Grading by this means was based on diameter of the hardened chorion, not of the yolk, but the two dimensions are probably correlated, and fry from the large eggs were significantly longer than those from the small eggs when all were preserved and stained about 4 months later. Meristic counts on samples (of 30, 35, and 37) from the three groups revealed no significant differences in numbers of vertebrae, dorsal rays, anal rays, or median pterygiophores.

Tåning (1952 p. 188) referring, without details, to experiments of *S. trutta*, wrote: "According to our investigations so far it may be said that the larger or smaller diameter of egg does not appear to have any certain influence on the number of vertebrae, and further that the size of the fry does not appear to mean anything either. It appears likewise that the number of vertebrae is the same in the first and last hatched fry of a batch of eggs."

The two preceding experiments dealt with salmonid eggs with large yolks, which might be supposed to lie above the critical limiting size hypothesized by Garside and Fry (1959). The eggs of *O. latipes* are in contrast very small, and yet they also failed to display a consistent correlation between egg size and vertebral count. The critical value, however, might be the ratio of the embryo size to the amount of yolk, concerning which we have no data.

If yolk size does not generally limit vertebral number, how are we to explain the intriguing results of Garside and Fry's investigation on twinned embryos? In the small-egged *S. fontinalis*, there was a significant negative correlation ($P < .01$) between the degree of separation of the twins and of the number of vertebrae formed in each twin. In the large-egged *S. namaycush* there was none. Yet, in *S. namaycush* eggs fertilized by *S. fontinalis* sperm, the same negative correlation existed as in the pure *S. fontinalis* cross, although it was less pronounced ($P < .05$). In other words, on the same (large) eggs, greater separation of twins produced greater reduction of vertebral count with one species of father but not with the other. Surprisingly, the reduction occurred only when the father was of the species that ordinarily has the lower vertebral count, so the result cannot be due to the *S. fontinalis* male having made a greater vertebral "demand" on a limited yolk supply. In any event, the factor that imposes a limit on vertebral count of twins seems to have been imparted in part from the male parent. What that factor is, remains quite unknown, but it cannot be solely the size of the egg.

Evidently the few studies based on egg measurements, and the more numerous but less satisfactory studies based on lengths of young fry, fail to support the hypothesis of a morphogenic mechanism linking vertebral count to egg size *amongst genetically similar fish*. On the other hand, there are many instances in which larger eggs do produce higher vertebral counts when comparisons are made *between genetically different units* within the same species, or genus, or family. For example, Garside and Fry (1959) found higher vertebral numbers in *Salvelinus namaycush* (which has a larger egg) than in *S. fontinalis*. That this difference was genetically determined by mechanisms other than egg size was demonstrated in a hybrid cross between an *S. namaycush* female and *S. fontinalis* male. The hybrids' vertebral counts lay between those of the parents and were strikingly lower than the pure *S. namaycush*, despite the fact that the hybrids were on the large *S. namaycush* eggs. In another series of hybridization experiments using sockeye salmon (the large-egged anadromous form of *O. nerka*, which has higher vertebral count) and kokanee (the small-egged lacustrine form), McCart (MS 1970) found that in one hybrid cross in which the eggs were from sockeye, the vertebral count was *lower* than in either parental form or in the reciprocal hybrid cross. He reports other crosses that gave opposing results, again demonstrating that determination of vertebral count is complex and not attributable solely to egg size.

In these and many other instances it is the species or race with the greater adult size that forms the higher vertebral count. *Salvelinus namaycush* adults

are larger than *S. fontinalis*; sockeye salmon are larger than kokanee. R. McV. Clarke has kindly provided us with calculations showing that in 10 races of Atlantic herring (*C. harengus harengus*) there is a striking correlation (r 0.85, $P < .01$) between average body length of spawning females and average vertebral count in each race (data from Schnakenbeck 1931, and Hempel and Blaxter 1967). There is an equally striking correlation (r 0.84) between average body length and average egg weight, but we suspect from artificial crossing (Hempel and Blaxter 1961) that vertebral difference between at least two of these races may be transmitted by the male and therefore cannot be attributable solely to egg size. There are many other examples (e.g., family Percidae (Bailey and Gosline 1955), and suborder Esocoidei) in which related species show a tendency for larger adult size to be associated with higher vertebral count. The larger species also often, but not invariably, lay larger eggs. Kyle's 1926 statement quoted in the Introduction may therefore be generally true when it is applied to different species, although not when it is applied to members of the same species in the same spawning group.

The selective values of vertebral differences in fish (as distinct from the embryonic mechanisms producing those differences) lie beyond the scope of this discussion. We are currently engaged in a general survey of the factors associated with vertebral variation. Tentatively, we suggest that amongst related species the vertebral count of each is adaptive to its average body size, and that the explanation may lie in the hydrodynamics of locomotion. If vertebral count has indeed been selected to match body size, then the reasons for the latitudinal clines often displayed by vertebral numbers may be associated with the striking latitudinal clines already demonstrated to occur in fish body sizes (Lindsey 1966). But regardless of what selective significance may eventually be attributed to vertebral count, the scattered evidence reviewed here suggests that morphogenic control of vertebral count does not reside in the egg size.

Acknowledgments

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A Comparison of Skin Tumors in Three Species of Flounders

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McArn, G. E., and S. R. Wellings. 1971. A comparison of skin tumors in three species of flounders. J. Fish. Res. Bd. Canada 28: 1241–1251.

A study of the natural history of skin tumors occurring on two species of Pleuronectidae was conducted in the waters of Bellingham Bay, Washington. Twelve collections at monthly intervals indicated that 4.8% of 1977 English sole (*Parophrys vetulus*) and 2.4% of 2946 starry flounders (*Platichthys stellatus*) were tumorous. Three types of tumors were observed, and were classified as angioepithelial nodules, epidermal papillomas, or angioepithelial polyps, according to nomenclature previously established by other workers. The angioepithelial nodule was found on fish in the 1st year of life but not on fish older than 1 year. Epidermal papillomas usually occurred on fish that were 1 year or older, as did the angioepithelial polyps. The incidence of tumor-bearing fish, and the average number of tumors on a single specimen was greatest during the 1st year of life and decreased progressively in older age-groups. Tumors were more likely to occur on the pigmented side than on the nonpigmented side of the fish. The results of this study were compared and contrasted with a similar disease previously described on *Hippoglossoides elassodon*, the flathead sole.

McArn, G. E., and S. R. Wellings. 1971. A comparison of skin tumors in three species of flounders. J. Fish. Res. Bd. Canada 28: 1241–1251.

Les auteurs ont étudié la biologie des tumeurs cutanées de deux espèces de pleuronectidés habitant les eaux de la baie de Bellingham, Washington. Douze échantillonnages mensuels révèlent que 4.8% des 1977 soles anglaises (*Parophrys vetulus*) et 2.4% des 2946 plies étoilées (*Platichthys stellatus*) examinées étaient affectées de tumeurs. Suivant la nomenclature déjà adoptée par d'autres chercheurs, on a reconnu trois types de tumeurs: nodules angioépithéliaux, papillomes épidermiques et polypes angioépithéliaux. Le nodule angioépithélial se rencontre chez les poissons de moins d'un an. Il est absent chez les poissons de plus d'un an. Les papillomes épidermiques et les polypes angioépithéliaux, par contre, se trouvent généralement chez les poissons âgés d'un an et plus. L'occurrence de poissons affectés et le nombre de tumeurs par individu atteignent un sommet durant la première année. Ils diminuent graduellement chez les poissons plus âgés. Les tumeurs ont tendance à se développer sur le côté pigmenté plutôt que sur le côté non-pigmenté du poisson. Les résultats de cette étude sont comparés et mis en contraste avec une maladie de même nature déjà décrite chez la sole à tête plate (*Hippoglossoides elassodon*).

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Numerous reports establish the existence of papillomas on a number of species of pleuronectid fishes (flounders) in the waters of Puget Sound and the San Juan Islands (Pacis MS 1932; Good MS 1940; Wellings et al. 1964; Wellings and Chuinard 1964; Chuinard et al. 1966; McArn et al. 1968). Other reports establish the existence of a similar if not identical lesion, hyperplastic epidermal disease, in this same group of fish collected at several other sites in the Northern Hemisphere (Johnstone 1912; Johnstone 1925; Thomas 1926; Schlumberger and Lucké 1948; Schlumberger 1957; Young 1964; Nigrelli et al. 1965; Kimura et al. 1967). These various reports suggest that this disease occurs frequently within this group of fish and may have circumpolar or world-wide distribution. The high frequency and wide distribution of the disease may be cause for concern since these fishes are consumed by both man and animals.

The natural history of skin tumors in flounders has been described in the flathead sole, *Hippoglossoides elassodon* (Chuinard MS 1966; Miller MS 1969), the English sole (*Parophrys vetulus*) (Cooper and Keller 1969), and in a preliminary survey report that documented the presence of tumorous fish in various areas in northern Puget Sound (McArn et al. 1968). The latter report indicated that in Bellingham Bay, Bellingham, Washington, two

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species of flounders, the English sole and the starry flounder (*Platichthys stellatus*), were frequently afflicted with tumors. Consequently, this area was chosen for an intensive study of the natural history of the disease. This allowed us to examine two previously unstudied populations and to determine whether or not the disease was similar to that found in the flathead sole population. The purpose of this report is to detail our findings and to compare the results with prior data on the flathead sole. Much of the data reported herein is contained in an unpublished thesis written by the senior author (McArn MS 1968).

Materials and Methods

Natural History

Collections

Fish in this study were collected in Bellingham Bay, Bellingham, Washington. Specimens were captured by means of a shrimp "try" net (0.5-inch stretch mesh in the bunt) operated from a fishing trawler, M.V. *Hydah*. A 15-ft beach seine (mesh size 0.25 inch) was used to collect early postmetamorphosis starry flounders along the southwest shore of the Nooksack Estuary.

Definite sampling stations were established in October 1966; these stations were sampled at monthly intervals thereafter for a period of 1 year (Fig. 1). The standardized collection procedures consisted of six 15-min tows of approximately 1600 yards each at depths of 4–7 fath. Water depths were obtained from soundings at mean low water on Coast and Geodetic Survey maps and by actual measurement with a bathythermograph. Temperature of the air, surface water, and bottom water was also measured. Collecting stations were reproducibly located by reference to shore topography and to the type of bottom material characteristic of that collection site.

Processing of tumor fish

The location of tumors on fish were tabulated according to quadrants as indicated in Fig. 2. All flounders collected were counted and examined. Fish possessing tumors were measured (total length and standard length) and the sex determined. The fish were grouped according to age by counting the number of annual growth rings on the auditory ossicles. The age-groups were defined as follows: age-group 0 — no translucent rings, indicating an age of 0–1 years; age-group 1 — one translucent ring, indicating an age of 1–2 years; age-group 2 — two translucent rings, indicating an age of 2–3 years; age-group 3 — three translucent rings, indicating an age of 3–4 years etc.

Histopathological Studies

Light microscopy

Tissue for light microscopy was fixed in Bouin's fluid or 10% formalin and embedded in paraffin. Five-micron sections were cut on a rotary microtome and stained by one or all of the following methods: hematoxylin and eosin, Masson's trichrome, periodic acid Schiff (PAS), Gridley's reticulum, Feulgen, Schorr's and Giemsa's stains (Anon. 1949).

Electron microscopy

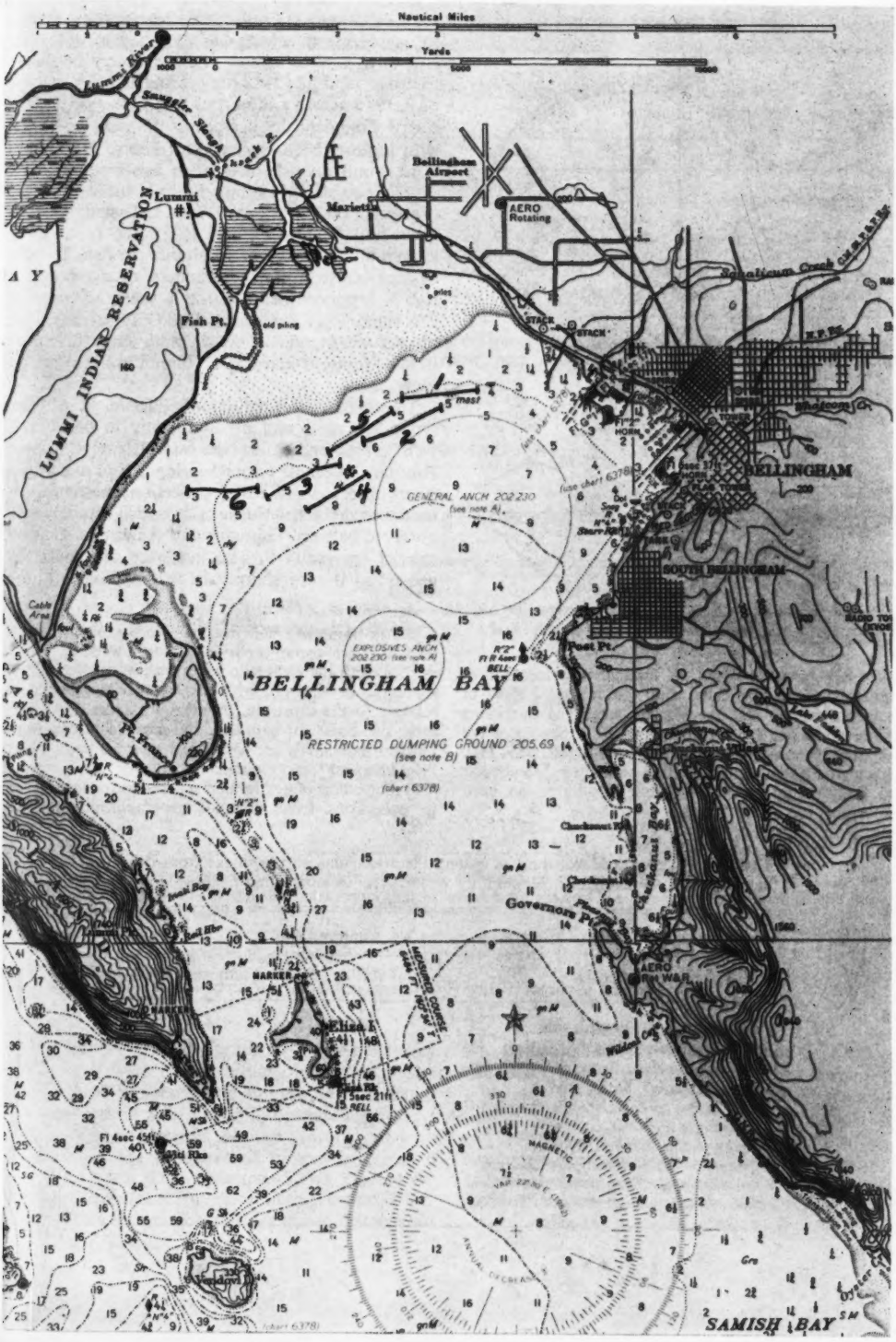
Tissue for electron microscopy was fixed in Lentz's fixative (Lentz and Trinkaus 1967) or 1.33% Os O_4 buffered with *s*-collidine (Bennett and Luft 1959), embedded in Swiss araldite (Luft 1961), and polymerized at 60 C for 7–10 days. Thick (1 μ) and thin (50–100 mμ) sections were cut with a Porter Blum MT-2 ultramicrotome. The thick sections were mounted on glass slides and stained with Richardson's stain (Richardson et al. 1960) for light microscopy. Thin sections were triple stained (Reynolds 1963), first with lead citrate for 5 min, then with saturated uranyl acetate for 1 min, and then once again with lead citrate for 8 min. Sections were viewed by means of an RCA EMU-3G electron microscope.

Results

All fishes and invertebrates collected in Bellingham Bay were examined for tumors and other surface abnormalities. Ten species of Heterosomata, including representatives of the families Pleuronectidae (flounders) and Bothidae (sanddabs), were represented; tumors were observed only on pleuronectids (Table 1). Of the 5240 pleuronectids collected, a total of 1977 English sole were examined, and 95 (4.8%) had skin tumors. Forty-two (1.5%) of the 2894 starry flounders collected had similar skin tumors, whereas 28 (54%) of the 52 starry flounders collected by beach seine were tumorous. The number of starry flounders collected by beach seine and from the fishing vessel therefore totalled 2946, of which 71 (2.4%) had tumors.

Fish with solitary and multiple tumors were observed in both of the above species. There was, however, a tendency for the number of tumors per fish to vary inversely with the age of the fish. English sole (Table 2) of age-group 0 had as many as 22 tumors on a single specimen. The average number of tumors per tumorous fish of age-group 0 was 4.0. English sole of age-group I had as many as 17 tumors on a single specimen for an annual average of 2.80 tumors per tumorous fish. No tumors were found on fish in age-group II or older.

FIG. 1. Sampling stations in Bellingham Bay, Bellingham, Washington.


Nautical Miles
Yards
Lummi River
Smuggler Slough
Lummi #1
Bellingham Airport
AERO Rotating
LUMMI INDIAN RESERVATION
Fish Pt.
Marietta
Squalicum Creek
STACK
BELLINGHAM
SOUTH BELLINGHAM
GENERAL ANCH 202.230
(see note A)
EXPLOSIVES ANCH 202.230 (see note A)
BELLINGHAM BAY
RESTRICTED DUMPING GROUND 205.69
(see note B)
(chart 6378)
Post Pt.
Governors Pt.
Cherry Point
Ft. Francis
Eliza I.
MARKER
AERO
SAMISH BAY

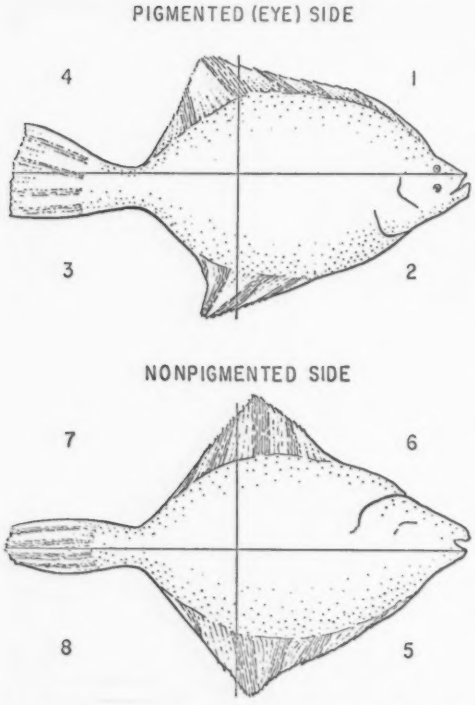


FIG. 2. Quadrants used to tabulate the location of tumors on the pigmented and nonpigmented sides of starry flounders and the English sole. The quadrants were established by drawing a line from the tip of the snout to the midpoint at the tip of the tail. This line, when divided in half by a line running perpendicular to the first, establishes the four quadrants on each side.

One right-handed (dextral[1]) starry flounder (Table 3), age-group 0, which was collected in 4–7 fath, had three surface tumors. Dextral starry flounders of age-group I had 1–18 tumors and a yearly average of 2.35 tumors per tumorous fish. Only two dextral starry flounders in age-group II were collected with tumors, both having two tumors. No tumors were found on fish older than age-group II.

Only two sinistral tumor-bearing starry flounders (Table 4) of age-group 0 were collected. One of these had 31 tumors, and the other 1, resulting in an annual average of 16 tumors per fish. Tumor-bearing sinistral starry flounders of age-group I had 1–7 tumors per fish for a yearly average of 2.1 tumors per tumorous fish. Only solitary skin tumors were observed in sinistral starry flounders of age-groups II and III; no tumors were found on older fish.

Tumor-bearing English sole (Table 5) of age-group 0 were found predominantly in collections 1–5, corresponding to the months of October–February. A single tumor-bearing fish of age-group 0 was observed in the May collection; this specimen represented the only instance in which a tumor was observed between February and August on English sole of age-group 0. The incidence of tumors in age-group 0 rose sharply in fish collected in the

[1]Starry flounders are unusual among pleuronectids in that after metamorphosis the fish may be either dextral, with the right side uppermost in relation to the substrate, or sinistral, with the left side uppermost in relation to the substrate. In either instance, the uppermost side possesses both eyes and most of the pigment. Dextral individuals are sometimes referred to as being "right-handed" and sinistral individuals "left-handed." The proportion of dextral and sinistral individuals varies in collections from different geographical locations.

TABLE 1. Incidence of skin tumors among Pleuronectidae and Bothidae from Bellingham Bay, Washington: 12 monthly collections, October 1966–October 1967.

Species	No. tumorous fish / Total no. fish	% tumorous fish
Parophrys vetulus, English sole	95/1977	4.80
Platichthys stellatus, starry flounder	71/2946	2.41
Psettichthys melanostictus, sand sole	2/61	3.28
Isopsetta isolepsis, butter sole	0/203	0
Citharichthys sordidus, Pacific sanddab	0/22	0
Lepidopsetta bilineata, rock sole	0/21	0
Glyptocephalus zachirus, rex sole	0/4	0
Hippoglossoides elassodon, flathead sole	0/3	0
Microstomus pacificus, dover sole	0/2	0
Atheresthes stomias, arrowtooth flounder	0/1	0

TABLE 2. English sole: tumor incidence according to age-group and location on specimen.

Age-group	No. fish with tumors	No. tumors on pigmented side	No. tumors on nonpigmented side	No. tumors on both sides	Total no. tumors	Range of no. tumors/fish	Avg no. tumors/fish
0	47	110 (57.9%)	57 (30.0%)	23 (12.1%)	190	1–22	4.04
I	48	66 (49.3%)	43 (32.1%)	25 (18.7%)	134	1–17	2.80
II	0	0	0	0	0	0	0
III	0	0	0	0	0	0	0
Total	95	176[a]	100[a]	48	324		

[a] X^2 determination: in determining the significance of the difference in occurrence of the tumors on the pigmented versus nonpigmented side of the fish, all tumors that involved both sides of the fish were eliminated, as the initial site of origin was unknown.

$$X^2 = \sum \frac{(176-138)^2}{138} + \frac{(100-138)^2}{138} = \frac{(38)^2}{138} + \frac{(-38)^2}{138} = 20.92;$$

X^2 exceeds the significant level of 0.1% with 1 degree of freedom.

TABLE 3. Starry flounder: tumor incidence according to age-group and location on specimen, dextral specimens only.

Age-group	No. fish with tumors	No. tumors on pigmented side	No. tumors on nonpigmented side	No. tumors on both sides	Total no. tumors	Range of no. tumors/fish	Avg no. tumors/fish
0	1	2 (66.6%)	0 (0.0%)	1 (33.3%)	3	1–3	3.0
I	17	18 (45.0%)	13 (32.5%)	9 (22.5%)	40	1–18	2.35
II	2	2 (50.0%)	0 (0.0%)	2 (50.0%)	4	2	2.0
III	0	0	0	0	0	0	0
Total	20	22[a]	13[a]	12	47		

[a] X^2 determination: in determining the significance of the difference in occurrence of the tumors on the pigmented versus nonpigmented side of the fish, all tumors that involved both sides of the fish were eliminated, as the initial site of origin was unknown.

$$X^2 = \sum \frac{(22-17.5)^2}{17.5} + \frac{(13-17.5)^2}{17.5} = \frac{(4.5)^2}{17.5} + \frac{(4.5)^2}{17.5} = 2.30;$$

X^2 determinations are less than the significant level of 5% with 1 degree of freedom.

month of September. Tumorous English sole of age-group I were found throughout the year. The incidence of tumors in the English sole increased from collections 2 through 5 (November–February) and decreased thereafter. No age-group I fish were found when the incidence of tumors in group 0 fish was highest (i.e., collections 1 and 12) and the highest incidence of tumors in age-group I occurred when the numbers of age-group 0 tumorous fish had declined markedly. Tumors in English sole older than age-group I were not observed.

Sinistral starry flounders of age-group 0 were seldom collected from the fishing vessel (Table 6), and only two of these were tumor bearing. However, beach seine collections during the months of July, August, and September established that numerous starry flounders of age-group 0 were present at several collecting sites in shallow (1 inch–3 ft)

TABLE 4. Starry flounder: tumor incidence according to age-group and location on specimen, sinistral specimens only.[a]

Age-group	No. fish with tumors	No. tumors on pigmented side	No. tumors on nonpigmented side	No. tumors on both sides	Total no. of tumors	Range of no. tumors/fish	Avg no. tumors/fish
0	2	19 (59.4%)	13 (40.6%)	0	32	1–31	16.0
I	16	11 (32.4%)	9 (26.5%)	14 (41.2%)	34	1–7	2.13
II	2	2 (100.0%)	0	0	2	–1	1.0
III	1	0	0	1 (100.0%)	1	–1	1.0
Total	21	32	22	15	69		

[a]X^2 determination: in determining the significance of the difference in occurrence of the tumors on the pigmented versus nonpigmented side of the fish, all tumors that involved both sides of the fish were eliminated, as the initial site of origin was unknown.

$$X^2 = \sum \frac{(32-27)^2}{27} + \frac{(22-27)^2}{27} = \frac{(5)^2}{27} + \frac{(5)^2}{27} = 1.85;$$

X^2 determinations are less than the significant level of 5% with 1 degree of freedom.

TABLE 5. English sole: tumor incidence by age-group and collection date.

Collection no.	Date	Age-group 0 (0–1 *year*)	I (1–2)	II (2–3)	III (3–4)	No. tumor fish age determined	Total fish with tumors	Total fish
				1966				
1	Oct. 3	15[a]	–	–	–	9	15	216
2	Nov. 4	4	3	–	–	7	7	41
3	Dec. 21	5	4	–	–	9	9	362
				1967				
4	Jan. 13	10	6	–	–	16	16	411
5	Feb. 7	3	20	–	–	23	23	332
6	Mar. 3	–	–	–	–	–	–	–
7	Mar. 31	–	2	–	–	2	2	7
8	May 5	1	2	–	–	3	3	81
9	June 2	–	2	–	–	2	2	91
10	July 18	–	5	–	–	5	5	183
11	Aug. 14	–	4	–	–	4	4	132
12	Sept. 14	9	–	–	–	9	9	121
Total		47	48	–	–	89	95	1977

% tumor incidence of total fish with tumor 4.81
% tumor incidence of age-determined tumor fish 4.50

[a]Six of the 15 fish died. Their ages were not determined by reading growth rings (annuli) on their ear ossicles. These six fish were age-grouped according to their total and standard lengths.

TABLE 6. Sinistral starry flounder: tumor incidence by age-group and collection date, sinistral specimens only.

Collection no.	Date	Age-group 0 (0–1 *year*)	I (1–2)	II (2–3)	III (3–4)	No. tumor fish age determined	Total fish with tumors	Total fish
				1966				
1	Oct. 3	–	1	–	–	1	1	160
2	Nov. 4	1	6	–	1	8	8	430
3	Dec. 21	–	–	–	–	–	–	32
				1967				
4	Jan. 13	–	–	–	–	–	–	128
5	Feb. 7	–	6	1	–	7	7	683
6	Mar. 3	–	1	1	–	2	2	380
7	Mar. 31	1	–	–	–	1	1	210
8	May 5	–	–	–	–	–	–	197
9	June 2	–	–	–	–	–	–	156
10	July 18	–	–	–	–	–	–	233
11	Aug. 14	–	1	–	–	1	1	138
12	Sept. 14	–	1	–	–	1	1	147
Total		2	16	2	1	21	21	2894

% total tumor fish .726
% total tumor age determined 0.726

protected waters. Thirteen (or 25%) of 52 starry flounders collected by beach seine (Table 8) in the Nooksack Estuary had tumors. Tumorous fish in age-groups I, II, and III were collected between October and March.

The dextral starry flounders in the collections (Table 7) displayed essentially the same characteristics as the sinistral group as regards the distribution of fish by age-group and collection date. Only one dextral tumor-bearing starry flounder of age-group 0 was collected in 5 fath; however, 15 (28%) of a total collection of 52 fish of the same species and age-group were collected (Table 8) in the Nooksack Estuary of Bellingham Bay by means of a beach seine during the months of July, August, and September. Tumor fish collected between October and May almost always were of age-group I or older. No dextral starry flounders were collected from June through September from the fishing vessel.

All four quadrants on a particular side of the afflicted fish were apparently about equally susceptible to tumor formation. However, tumors occurred more frequently on the pigmented side, rather than on the nonpigmented side of the fish. The chi-square test indicated that this tendency was significant at the $P = 0.1\%$ level with 1 degree of freedom for the English sole (Table 2).

In this study, right- and left-handed starry flounders collected from the fishing vessel showed no significant tendency for the lesions to occur on either side of the fish (Tables 3 and 4). Starry flounders collected by beach seine, however, showed a statistically significant tendency for more tumors to occur on the pigmented side than on the nonpigmented side (Table 8). This tendency was significant at the $P = 5\%$ level with 1 degree of freedom. In another study, the predominance of tumors on the pigmented side was of greater statistical significance (McArn MS 1968).

Skin tumors

Normal skin and skin tumors from English sole and starry flounders were identical to previous descriptions by both light and electron microscopy, and were also indistinguishable from tumors described in the flathead sole (Wellings et al. 1964; Wellings et al. 1965). The same three histopathologic tumor types occurred: angioepithelial nodules (AEN), epidermal papillomas (PAP), or angioepithelial polyps (AEP).

The AEN were the smallest lesions encountered, and generally were on the smallest and youngest fish. These tumors measured 1–4 mm, were smooth surfaced, and were red to pink in color. Micro-

TABLE 7. Dextral starry flounder: tumor incidence by age-group and collecting date, dextral specimens only.

Collection no.	Date	Age-group 0 (0–1 *year*)	I (1–2)	II (2–3)	III (3–4)	No. tumor fish age determined	Total fish with tumors	Total fish
				1966				
1	Oct. 3	–	2[a]	–	–	2	4	160
2	Nov. 4	–	4	–	–	4	4	430
3	Dec. 21	–	–	–	–	–	–	32
				1967				
4	Jan. 13	–	2	–	–	2	2	128
5	Feb. 7	–	1	–	–	1	1	683
6	Mar. 3	1	3	1	–	5	5	380
7	Mar. 31	–	1	2	–	3	3	210
8	May 5	–	1	1	–	2	2	197
9	June 2	–	–	–	–	–	–	156
10	July 18	–	–	–	–	–	–	233
11	Aug. 14	–	–	–	–	–	–	138
12	Sept. 14	–	–	–	–	–	–	147
Total		1	16	4	–	19	21	2894

% tumor incidence, total tumor fish 0.723
% tumor incidence, age-determined fish 0.656

[a]Two of the four fish died. Their age was not determined by reading growth rings (annuli) on their ear ossicles. These two fish were age-grouped according to their total and standard lengths.

TABLE 8. Starry flounder: occurrence of skin tumors on pigmented versus nonpigmented sides, all specimens of age-group 0, both sinistral and dextral, collected by beach seine.[a]

No. fish with tumors	No. tumors on pigmented side	No. tumors on nonpigmented side	No. tumors on both sides	Total no. of tumors	Range of no. tumors/fish	Avg no. tumors/fish
28	42 (62.7%)	24 (35.8%)	1 (1.5%)	67	1–14	2.39

[a]X^2 determination: in determining the significance of the difference in occurrence of the tumors on the pigmented versus nonpigmented side of the fish, all tumors that involved both sides of the fish were eliminated, since the initial site of origin was unknown.

$$X^2 = \sum \frac{(42-33)^2}{33} + \frac{(24-33)^2}{33} = \frac{81}{33} + \frac{81}{33} = 2.45 + 2.45 \text{ or } 4.90;$$

X^2 determination exceeds the significant level of 5% with 1 degree of freedom.

scopically, they were composed mostly of vascular connective tissue of dermal origin covered by a slightly thickened hyperplastic layer of epidermal cells, some of which showed enlarged nuclei and nucleoli. Mucous cells were decreased in number or absent.

The typical PAP are large cauliflowerlike lesions (1–4 cm in diameter), grey to brown to black in color. They are characterized by a marked papillomatous proliferation of the epidermis, supported by a fine, poorly vascularized connective tissue stroma. At least some tumors progressively enlarge in the laboratory, but metastasis has never been observed. Similar tumors have been previously described on *Psettichthys melanostictus* (Wellings et al. 1964; Nigrelli et al. 1965), *Parophrys vetulus*

(Pacis MS 1932; Good MS 1940; McArn et al. 1968; Cooper and Keller 1969), *Limanda limanda* (Johnstone 1912), *Glyptocephalus zachirus* (Wellings et al. 1964), and the starry flounder (McArn MS 1968).

The AEP are not frequently found on either the starry flounder or the English sole. In contrast to most epidermal papillomas, the large, raised, soft angioepithelial polyps are sharply demarcated from the surrounding skin at the periphery of the tumor. Microscopically, the bulk of the tumor consists of vascular connective tissue surfaced by normal or slightly hyperplastic epidermis, with or without mucous cells. Frequently, some portions of the epidermis possess cells that are enlarged and resemble those cells typically found in the thickened epidermal folds of the papilloma. The angioepithelial polyps occur in fish of age-group I or older.

Electron microscopy revealed the same distinctive, unidentified cell type ("X-cell") described by Brooks et al. (1969) in the tumors of the flathead sole. Inasmuch as the morphology of the present tumors is identical to previous descriptions, photographic confirmation was deemed unnecessary, and has been omitted from the report.

The light microscopy and ultrastructure studies revealed "X-cells" in the dermis of the AEN and in the dermis and epidermis of the PAP, and the AEP. Unlike normal skin cells or hematopoietic components these hypertrophic cells increase in number as the tumor progresses through the various stages of development from the AEN to the PAP. The "X-cells" are found first as small cells in the dermis of the early AEN and as large hypertrophic cells in both the dermis and epidermis of the PAP and AEP. Distinctive features of this hypertrophic cell include: (*a*) a large nucleus and prominent nucleolus; (*b*) numerous dense cytoplasmic granules; (*c*) a lack of intercellular bridge formation with surrounding epidermal cells, which form connections with other epidermal cells; (*d*) a thickened extracellular coat, which is thicker on cells found in the dermis than in the epidermis; (*e*) the "X-cells" are not uncommonly observed in groups of 2, 3, and 4.

Discussion

The present study outlines in detail the natural history of skin tumorigenesis in the English sole and the starry flounder found in Bellingham Bay, Washington. A preliminary survey report (McArn et al. 1968) of several collecting sites in lower Puget Sound indicated that Bellingham Bay was a productive and therefore favorable site in which to conduct an extensive study of this disease process observed in the above two species with an apparently similar disease previously described in the flathead sole collected in East Sound of Orcas Island (Wellings et al. 1965).

The results of this study are comparable to those of the flathead sole study in the following ways.

A similar yearly cycle of tumorigenesis occurs in the English sole, the flathead sole, and the starry flounder populations.

The histopathology of the skin tumors of the starry flounder and the English sole is essentially identical to that of the tumors of several other species of flounder, including the flathead sole (Wellings et al. 1965).

The tumors are of the same morphologic types: angioepithelial nodules, epidermal papillomas, and angioepithelial polyps.

The three tumor types appear within the same age-groups (year-classes) and in the same sequential manner as was observed in the flathead sole.

The AEN or early lesion is found predominantly on fish in the 1st year of life.

Fish of age-group I or older are afflicted with the typical PAP, or less frequently, with the AEP.

The data support the previously proposed histogenetic relation between the AEN, PAP, and AEP, found in populations of flathead sole (Wellings et al. 1965) for the following reasons: firstly, the nodules are more frequent on the smallest and youngest fish of the two species studied, and the PAP and AEP are observed on older fish. Secondly, numerous transitions between these three tumor types are noted, both grossly and microscopically, during the winter months. Thirdly, AEN on English sole are observed to develop through various transitional forms into the mature PAP under laboratory conditions (G. McArn unpublished data). These same transitions were also observed under laboratory conditions in the flathead study (Chuinard MS 1966).

Field observations of gravid females and ripe males of both the starry flounder and the English sole indicate that, like the flathead sole, spawning occurs in the months of March, April, and May. Tumorous postmetamorphosis starry flounders and English sole of age-group 0 were collected in the months of July–October. The correlation of tumor type and age distribution is similar in all three species of fish. Only the youngest English sole and starry flounders had the AEN, whereas fish less than 1 year of age had the transitional lesions. The PAP were almost exclusively limited to fish that were 1 year or older.

Application of the chi-square test indicates that the predominance of tumors on the pigmented (eye) side of age-group 0 fish (both dextral and

sinistral starry flounder collected by beach seine, and English sole, collected from the vessel) were statistically significant. The pelagic pleuronectid larvae are bilaterally symmetrical prior to metamorphosis, and the right and left sides are identical except that they are mirror images of each other. During metamorphosis the larvae become asymmetrical with their pigmented (eye) side uppermost with respect to the substratum, leaving the nonpigmented (blind) side lowermost with respect to the substratum. As has been pointed out previously (Wellings et al. 1964), the difference in attack rate on the two sides suggests that the tumorigenic event, or events, may occur some time after metamorphosis, since prior to that time the two sides are essentially identical. Since starry flounder metamorphosis occurs when the larvae reach about 10.0 mm in standard length, it would be expected, therefore that the tumorigenic event(s) might occur some time after the larvae reach 10.0 mm and before they reach 30.0 mm in standard length, because tumors are known to occur on fish 30.0 mm long. This would narrow the interval of tumorigenesis in this species to a 2–3-month period during the summer months.

The same line of reasoning applies to English sole of age-group 0. Since we were unable to locate their "nursery," the fish were older when collected exclusively from the vessel. The AEN are most numerous during the months of September and October and decrease in number in the following months. As the number of AEN decreases, the number of transitional tumors and PAP increase, suggesting, as did the flathead study, that the AEN evolve into PAP. The average number of tumors per fish in English sole of age-group 0 was less than that in starry flounders of the same age-group.

It is not known whether fish die of the disease, or recover, or whether either event occurs. It seems reasonable to assume that fish with massive, progressive lesions are less maneuverable, possibly less successful in obtaining food, and more susceptible to predation than are normal fish. Field observations indicate that tumorous fish may be less robust and succumb to the trauma of the collection net much more readily than healthy normal fish. The high incidence of tumors in age-group 0 starry flounder (54%) is not reflected on older age-groups and suggests that these fish are removed from the population. Morphometric and survival studies of the flathead sole indicate that tumor-bearing fish are significantly smaller and have a shorter life expectancy than normal fish after their 1st year of life (Miller MS 1969). First-year weight, length, and life expectancies of normal and tumor fish are comparable.

Another point of similarity between the flathead sole study and the present study is the presence of an identical unknown cell type located in the dermis and epidermis of these tumors. The question of whether the cell is a transformed fish cell or an exogenous unicellular parasite remains unanswered. The lack of similarity between the "X-cell" and normal cell components does not rule out the possibility of transformation nor does the suggested inflammatory reaction in the dermis of the AEN indicate that the cell is of exogenous origin. At this time we can only cite the similarities in both studies and suggest that these two alternatives are possibilities.

The results of this study are at variance with the flathead data in the following ways.

Starry flounders in age-group I or older generally had solitary PAP. As mentioned in the results, chi-square tests indicated that tumor distribution according to side (pigmented vs. nonpigmented) was not significantly different. Earlier work, however, strongly suggested that there was a preferential tendency for tumors to occur on the pigmented side (McArn et al. 1968). The flathead study indicated that fish 1 year or older had multiple tumors and that there was a tendency for these tumors to occur on the pigmented side of the fish (Wellings et al. 1964).

English sole, collected from the vessel, were first observed to have tumors at approximately 5–6 months old. Very few starry flounders collected from the *Hydah* were in the same age-group, and fewer still were afflicted with the AEN. Essentially all normal and tumorous flathead and English sole specimens were collected from motor vessels in deeper water and few if any were collected from the beaches. Beach seine collections near the mouth of the Nooksack River were in sharp contrast to collection data obtained from the fishing vessel. Numerous starry flounder of age-group 0, measuring 1–4 cm in total length, were collected from the beaches. The incidence of AEN in these group 0 starry flounders was very high; 54% of these fish had one to several AEN. This incidence exceeds that found at any other collecting site or in any other age-group or species of fish so far studied by the authors. This "nursery" area for the very young fish (immediately after metamorphosis) consisted of shallow, inshore, protected mud–sand flats characterized by higher water temperature and often relatively low salinity. These hydrographic features could be related in some way to the prevalence of the disease at this collecting site. Monogenetic trematode infestation and tumor formation

exist concurrently. This circumstance is worthy of more intensive study to determine if trematode infestation had any relation to tumorigenesis.

English sole were not observed in the Nooksack estuary, nor were we able to locate the nursery of this species. The flathead sole nursery to date has yet to be found.

The high incidence of tumors in very young populations is contrary to most spontaneous tumorigenic processes and suggests that an infectious agent may be operative.

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Antibody Production and Immune Responses of Rainbow Trout and Coho Salmon to *Chondrococcus columnaris*

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FUJIHARA, M. P., AND R. E. NAKATANI. 1971. Antibody production and immune responses of rainbow trout and coho salmon to *Chondrococcus columnaris*. J. Fish. Res. Bd. Canada 28: 1253–1258.

Active immunity to *Chondrococcus columnaris* disease was established in coho salmon (*Oncorhynchus kisutch*) approximately 3 months old by oral vaccination with heat-killed cells. Average agglutinating titers were 1:17 for control survivors and 1:168 for the orally vaccinated salmon. When rainbow trout (*Salmo gairdneri*) approximately 3 months old were exposed in a trough to *C. columnaris* present as a natural contaminant in Columbia River water, 52% of the fish died during the first 6 weeks of the test. Fish that survived the exposure were generally resistant to subsequent challenge. Many age 1+ rainbow trout that survived the *C. columnaris* infection as juveniles, when sampled after several months of reexposure to the disease, were immune carriers of the pathogen and developed agglutinating antibody titers ranging from 1:80 to 1:640. Titers up to 1:5120 were developed in disease-free yearling rainbow trout by parenteral vaccination.

FUJIHARA, M. P., AND R. E. NAKATANI. 1971. Antibody production and immune responses of rainbow trout and coho salmon to *Chondrococcus columnaris*. J. Fish. Res. Bd. Canada 28: 1253–1258.

Un vaccin oral à base de cellules tuées par la chaleur a produit chez des saumons coho (*Oncorhynchus kisutch*) âgés d'environ 3 mois une immunité active contre *Chondrococcus columnaris*. Les titres moyens d'agglutination étaient de 1:17 pour les survivants témoins et 1:168 pour les saumons vaccinés. Lorsque des truites arc-en-ciel (*Salmo gairdneri*) âgés d'environ 3 mois furent exposées en vivier à *C. columnaris*, un contaminant naturel des eaux du fleuve Columbia, 52% des poissons moururent durant les 6 premières semaines de l'expérience. En général, les survivants ont résisté à des contacts subséquents. Plusieurs truites âgées de plus d'un an qui avaient résisté, comme juvéniles, à l'infection par *C. columnaris* se sont avérées porteurs immunisés lorsqu'examinées après plusieurs mois de réexposition au microbe pathogène. Les truites avaient formé des anticorps agglutinants de titres allant de 1:80 à 1:640. La vaccination parentérale de truites arc-en-ciel d'un an noncontaminées a produit des titres allant jusqu'à 1:5120.

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THIS paper describes the results of laboratory exposure of rainbow trout (*Salmo gairdneri*) approximately 3 months old, to *Chrondrococcus columnaris*, and demonstrates the development of natural immunity and immune carriers among the survivors. In addition, the development of agglutinating antibodies in rainbow trout approximately 1 year old through parenteral vaccination, and an immune response in coho salmon (*Oncorhynchus kisutch*) approximately 3 months old by oral vaccination with heat-killed *C. columnaris* cells, are described.

The mortality of rainbow trout approximately 3 months old and chinook salmon (*Oncorhynchus tshawytscha*) of a similar age in our hatchery has been directly associated with: population density of fish, the start of warm river temperatures, and isolation of large numbers of *C. columnaris* organisms from the trough river water and fish (Fujihara et al. 1964). Diseased fish have been found in the rearing troughs concurrently with isolation of the pathogen from river fish, and when river temperatures increase to 10–12.8 C the disease can be controlled in the laboratory by reduction of the

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water temperature; however, a practical means of preventing the disease does not exist.

Duff (1942) demonstrated the feasibility of orally vaccinating cutthroat trout (*Salmo clarki*) against *Aeromonas salmonicida* (furunculosis disease). Oral and intraperitoneal vaccination of brook trout (*Salvelinus fontinalis*) (Snieszko and Friddle 1949) and oral vaccination of coho salmon (Spence et al. 1965; Klontz 1967, 1968) against furunculosis have been investigated but the results have been inconclusive. Various antigens of *A. salmonicida* are being investigated by Anderson (1969) to determine which of the antigens are of primary importance in inducing protective immunity in rainbow trout.

Materials and Methods

Exposure of Fish to C. columnaris

Sixteen hundred rainbow trout approximately 3 months old were exposed to *C. columnaris* occurring naturally in hatchery river water. The course of mortality was followed daily and all fish that died during the experiment were examined for visible necrotic surface lesions normally associated with *C. columnaris* disease in young salmonids. Samples from surface lesions, if present, and gills of all dead fish were surface-plated on solid medium at *p*H 7.3 containing 1% Noble's agar, 0.4% Bactotryptone, and 0.04% Bacto-yeast extract. The plates were incubated at 25 C for 48 hr, at which time they were examined for typical *C. columnaris* colonies. To evaluate the disease incidence in the trough during the 6-month test period, 25 live fish were randomly sampled at weekly intervals and gills and lesions, if present, cultured for *C. columnaris*.

Determination of Immune Carrier State

To determine if yearling rainbow trout that had survived exposure to *C. columnaris* as juveniles were acting as carriers of the disease, 10 trout were individually isolated in jars supplied with river water in August 1964, 1 month following the normal occurrence of the disease in our troughs. At 5–10-day intervals, single fish were transferred to separate jars containing 1 liter of sterile river water. The fish were removed after 1 hr and returned to the individual isolation jars, and the disease organisms in the water counted.

The carrier aspect was also investigated by sampling river water for 5 months at 5–10-day intervals from the outlet of a trough holding 25 age 1+ survivors of the previous year.

Twenty plate counts were made during each sampling of the water from the containers that held the isolated fish and from the trough. At each sampling, four 0.25-ml aliquots from each of five 1-ml samples were surface-plated on the solid-growth medium described above. Plate counts of the test water provided estimates of *C. columnaris* released by individual fish and by a group of fish.

Preparation of Agglutinating Test Antigen and Parenteral Vaccine

Test and immunizing antigens were prepared by inoculating liquid medium containing 0.4% Bactotryptone and 0.04% Bacto-yeast extract at *p*H 7.3 with lyophilized virulent *C. columnaris* cells. The organisms were grown at 25 C for 20 hr on a rotary shaker at 200 rpm. The cells were then washed three times with sterile physiological saline and harvested by centrifugation. The cells for the test antigen were suspended in physiological saline at a concentration of approximately 2.5×10^8 cells/ml and those cells for the parenteral vaccine in saline or 25% Freund's complete adjuvant (or both) at a concentration of approximately 1.6×10^8 cells/ml. Prior to adjusting the concentrations of the test antigen and vaccine, the cells were inactivated in a water bath at 65 C for 1 hr.

Determination of Agglutinating Antibody Titers

A standard tube agglutination test described by Conn (1957) was used to determine the titers of agglutinating antibodies to *C. columnaris* in fish sera. The tube test was modified to use a volume of 0.45 ml physiological saline and 0.05 ml of fish sera before dilution. Serial dilutions of 0.25 ml were made from the original saline–serum solution and 0.25 ml of the test antigen (heat-killed suspension of *C. columnaris* cells, 2.5×10^6 cells/ml) was added to each of the dilution tubes giving final dilutions of 1:20–1:5120. The tubes were incubated at room temperature for 1 hr and at 5 C for 20 hr at which time they were examined for agglutination. The highest dilution in which macroscopic agglutination occurred was recorded as the serum titer value.

Since repeated blood samples from the same fish were required, the volume of blood removed per sampling was kept at 0.5 ml. Blood samples were drawn by dorsal aorta punctures (Schiffman 1959) without the use of anticoagulants, refrigerated for 0.5 hr to reduce the formation of serum clots, and centrifuged in 0.5 ml disposable plastic tubes. The serum from each blood sample was pipetted into similar disposable tubes and refrigerated until agglutination tests were performed.

Preparation of Oral Vaccine

Heat-killed vaccine was prepared as described above and sonicated extracts of the vaccine were prepared by continuous disruption of the cells in a Bronson sonifier with an energy input of 130 w for 10 min. The container and cells were cooled in a water jacket connected to a water supply during sonification.

Oregon moist pellet, a commercially available fish food, was used as a filler for the vaccine. The food was dried overnight at 60 C and 750 g dry weight of the food was sprayed with 250 ml (approximately 1.6×10^8 cells/ml) of the heat-killed or sonically disrupted cells, giving a final concentration of approximately 4×10^7 cells per gram wet weight of food. The addition of the cell suspension in physiological saline returned the fish food to approximately the original moisture content of the commercially available pellet. The vaccine-

mixed food was kept refrigerated until used to retard fungus growth.

Vaccination of Fish

One-year-old rainbow trout, reared in a dechlorinated water supply and with no history of exposure to *C. columnaris*, were used for all parenteral vaccination studies. These fish were maintained during the test in dechlorinated water to prevent accidental infection. Subcutaneous and intraperitoneal vaccinations were performed by injection of 0.1 ml of heat-killed cells in a physiological saline suspension containing approximately 1.6×10^7 cells or a similar suspension of cells in 25% Freund's complete adjuvant. The fish were injected with the vaccine at weekly intervals for the first 4 weeks and at 2-week intervals until the last injection at 12 weeks. Controls were injected at the same intervals with 0.1 ml saline or 0.1 ml saline containing 25% Freund's complete adjuvant.

In another experiment, control and experimental year-old rainbow trout vaccinated intraperitoneally as described above were placed in a trough supplied with Columbia River water. The water input was connected to a 5-gal-per-min water supply, which contained *C. columnaris* infected rainbow trout approximately 4 months old. This permitted us to utilize *C. columnaris* released into the water under conditions much higher than would normally occur in the river. The experiment was initiated in August 1967, 1 month after the disease was well-established in the juvenile trout population. Antibody production was assayed at monthly intervals for 12 months.

Rainbow trout approximately 3 months old, reared in our hatchery in dechlorinated water, and coho salmon of a similar age obtained from the Naches Hatchery, Bureau of Sports Fisheries and Wildlife, Naches, Washington, were used for the oral vaccination studies. Both species had no history of *C. columnaris* exposure when the test was initiated. Oral vaccination was accomplished by feeding vaccine-treated food to 600 rainbow trout and to 600 coho salmon closely confined in $1 \times 1 \times 5$-ft troughs supplied with Columbia River water. Sonically disrupted cells were fed to rainbow trout and heat-killed cells to coho salmon. A single daily saturation feeding of the appropriate oral vaccine was based on river water temperature in the troughs and weight and number of fish according to established hatchery feeding procedures. The daily feeding of vaccine was continued throughout the 7-week test period. Controls were fed similarly with untreated food. Feeding of the vaccine-treated food was initiated in May 1968, 2 months prior to the predictable occurrence of the disease in our hatchery in July.

Results

The mortality of juvenile rainbow trout in our troughs occurred at river water temperatures above 15 C (Fig. 1). The initial increase in the percentage of live fish harboring *C. columnaris* was associated with a high mortality (28%). Decreases in mortality and *C. columnaris* incidence were followed by a second increase in percentage of fish harboring the pathogen, though not accompanied by increased mortality. During the two periods of peak incidence of live fish with isolates, 300–400 organisms/ml were isolated from the trough water. The rapid decrease of mortalities after the initial peak at 4 weeks and the continued presence of large numbers of organisms on the fish and in the trough water suggested not only the possibility of an immune response against *C. columnaris* but of an immune-carrier state.

Chondrococcus columnaris-associated mortality was observed among 832 of the 1600 rainbow trout during the initial 6 weeks of the experiment (July through the middle of August 1964). During this interval 65% (541) of the dead fish had visible necrotic surface lesions encircling the base of one or more of the fins, or gill lesions, or both areas. In both types of lesions, myriads of *C. columnaris* organisms were isolated.

When individual age 1+ rainbow trout that survived *C. columnaris* disease as juveniles were isolated in river water (18–20 C) and subsequently placed in 1 liter sterilized river water at similar temperatures for 1 hr, cyclic patterns of *C. columnaris* release were observed (Fig. 2). Some of the trout demonstrated three cycles of release with as many as 5×10^3 organisms/ml released in 1 hr, whereas another group showed one cycle over a 28–30-day period without further release of organisms. However, when these fish were returned to a trough holding other age 1+ trout survivors in Columbia River water, reexposed to the disease and subsequently retested, similar large release values were again observed with an eventual drop to zero release. Other isolated fish showed no *C. columnaris* release into the water. These studies indicate that some of the rainbow trout may have become immune carriers of the disease.

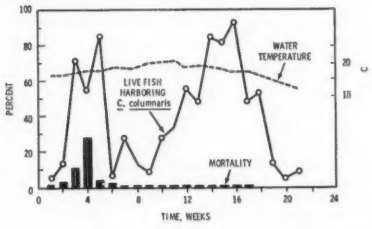

FIG. 1. Weekly mortalities among 1600 juvenile rainbow exposed to Columbia River water from July through December 1964, and percentages of live fish, in weekly samples of 25 fish, from which *C. columnaris* was isolated.

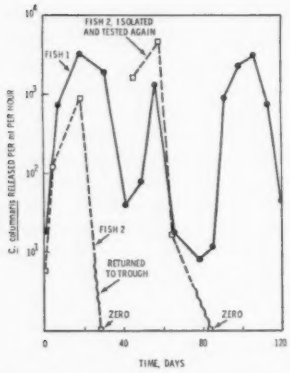

FIG. 2. Pattern of *C. columnaris* release for two disease-carrier yearling rainbow trout isolated for 1-hr intervals in 1 liter of sterile Columbia River water from August through November 1964.

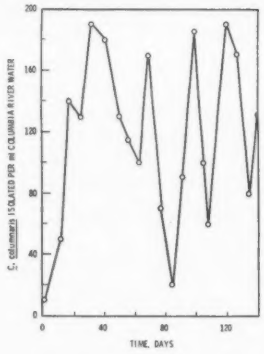

FIG. 3. Pattern of *C. columnaris* release for 25 yearling rainbow trout held in a trough with Columbia River water from July through November 1964.

A similar cycling of bacterial release was observed in a trough holding 25 age 1+ rainbow trout survivors of *C. columnaris* disease (Fig. 3). Although the release patterns of individual fish were masked, at the end of 140 days, 10 of these fish were releasing organisms and 15 were not. The peak cyclic releases of approximately 190 organisms/ml of water obtained at the trough outlet were considerably lower than those shown in Fig. 2, because these samples were taken from a trough with a constantly flowing water supply at 4 gal/m. The incoming river water supply was consistently negative. Although *C. columnaris* must have been entering the trough, the water volume sampled during each interval (5 ml) was probably too small to pick up the pathogen. No mortalities occurred during the test period.

When agglutinating antibody production in these same 25 rainbow trout was examined during May 1964, 2 months before the second annual exposure to *C. columnaris*, residual titers ranging from 1:40 to 1:160 (avg 1:90) were observed in 22 of the 25 fish. The majority of these fish retained *C. columnaris* antibodies during the winter, when probability of exposure and infection was slight. However, when the fish were examined in October after a 4-month exposure to the pathogen, an increase in agglutinating antibodies was observed with titers ranging from 1:80 to 1:640 (avg 1:204). These observations provide additional support for the contention that the presence of antibodies against *C. columnaris* in the blood sera of rainbow trout provides some protection against the disease.

Further support for an association between survival of fish and antibody production was obtained by vaccinating rainbow trout, approximately 1 year old, that had no history of the disease with heat-killed suspensions of *C. columnaris* (Table 1). These fish were reared in dechlorinated water and had no detectable antibodies against the disease. Antibody production was apparent

TABLE 1. Maximum agglutinating titers induced at weekly intervals in yearling rainbow trout vaccinated intraperitoneally and subcutaneously with heat-killed *C. columnaris* antigen and antigen mixed with Freund's Complete Adjuvant.

Injection of antigen	No. fish	Agglutinating titers at weeks										
		0	1	2	3	4	5	6	7	8	10	12
Intraperitoneal	20	0	0	20	40	80	320	320	640	640	640	640
Intraperitoneal plus 25% adjuvant	25	0	20	40	80	160	320	640	640	1280	2560	5120
Subcutaneous	25	0	0	40	80	80	320	320	160	640	320	640
Subcutaneous plus 25% adjuvant	20	0	20	80	160	320	320	640	640	1280	2560	2560

within 2 weeks after vaccination with either heat-killed cells or cells plus 25% Freund's complete adjuvant. Sham-vaccinated controls (0.85% physiological saline) did not induce any detectable antibodies during the experiment. Average titers of 1:480 (range 1:160–1:640) were observed in rainbow trout injected with heat-killed organisms without adjuvant in 12 weeks; however, higher average titers of 1:900 ranging from 1:320 to 1:5120 were observed in 12 weeks in those fish receiving heat-killed cells plus adjuvant. The difference in agglutinating values between the subcutaneous and the intraperitoneal experimental groups are probably of doubtful significance. Although none of the fish were lost during the experiment, the use of subcutaneous vaccination with the adjuvant was found undesirable because of large "boils" that developed at the site of injection.

When test antigens from avirulent and virulent *C. columnaris* organisms isolated from coarse fishes were checked against sera from these vaccinated fish, similarly high agglutinating values were observed. However, no cross reaction was observed against *Escherichia coli*, *Aeromonas salmonicida*, or several unidentified bacterial contaminants isolated from coarse fishes. These results suggest that the antibodies are probably specific for *C. columnaris* antigens; however, the possibility of a nonspecific response cannot be overlooked.

The annual changes in antibody titers in 15 control and 15 rainbow trout, approximately 1 year old, vaccinated intraperitoneally, demonstrate the value of vaccination and the faster development of antibodies that may protect the fish against *C. columnaris* disease (Fig. 4). Agglutinating titers of the controls increased from zero during August 1967, to an average peak of 1:400 (range 1:320–1:640) in November during the first 4 months and decreased to an average of 1:10 (range 0–1:20) during the winter and early spring, when fish were not exposed to *C. columnaris*. A gradual increase in average titers was observed from May 1968, with the start of warmer temperatures and reexposure of the fish.

The initial decrease (Fig. 4) in average titer of the vaccinated fish from 1:1000 (range 1:640–1:1280) in August to 1:650 (range 1:320–1:1280) in November, despite *C. columnaris* challenges up to 15 organisms/ml released from the juvenile trout, can probably be explained by the exceptionally high agglutinating antibodies that were induced prior to introduction of the fish into river water, and suggests that the challenge was insufficient to increase antibody development until titer values dropped to a lower level. Three of the 15 controls died during the first 3 weeks of the test from *C. columnaris* infection of the kidney. No mortalities occurred in the vaccinated fish. The comparatively high residual antibodies observed in the vaccinated trout during the early winter months and the rapid increase in titer after reexposure imply that vaccination and the faster development of antibodies may protect fish against *C. columnaris* disease.

Resistance to *C. columnaris* disease was not developed in rainbow trout approximately 3 months old that were fed sonically disrupted cells mixed into the fish food. Seventeen weeks after the test was initiated, mortality for the 600 control trout was 27% as compared to 19% for the vaccine-fed trout.

Chondrococcus columnaris-associated mortality was first observed among 600 control coho salmon 2 months after the start of the experiment (Fig. 5), the infection having arisen from organisms that entered the troughs from the river. The daily feeding of heat-killed *C. columnaris* cells mixed in fish food delayed mortality for 4 weeks and reduced mortality rates throughout the 17-week test period.

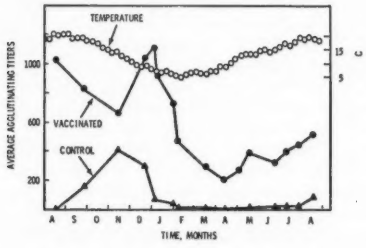

FIG. 4. Monthly average agglutinating titers for 15 control and 15 experimental year-old rainbow trout vaccinated intraperitoneally with heat-killed *C. columnaris* cells and exposed from August 1967 to August 1968 to Columbia River water contaminated with organisms released from 4-month-old rainbow trout.

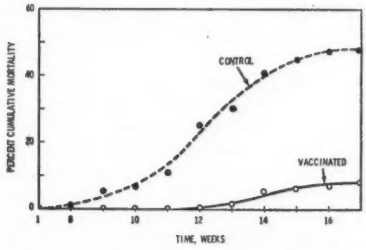

FIG. 5. Cumulative weekly mortalities among 600 controls and 600 juvenile coho salmon fed daily for 17 weeks (May–October 1968) with heat-killed *C. columnaris* cells mixed in fish food.

Though the fish were too small (6–10 cm) to obtain blood samples for determination of agglutinating antibody production during the test, average titers for pooled blood sera from two to three fish each at the completion of the test were 1:17 (range 0–1:40) for controls and 1:168 (range 1:40–1:320) for the vaccinated fish. Final mortality for controls was 48% as compared to 8% for the vaccine-fed coho salmon, indicating the establishment of immunity to the disease. Plate counts of the trough water showed less than 1 *C. columnaris* isolate/ml at 8 weeks when the first control fish mortality was observed. However, at the completion of the experiment, the numbers of *C. columnaris* isolates were approximately 900/ml in the control trough water and 300/ml in the experimental trough. The release values are higher than fish would normally encounter, and indicate that heat-killed *C. columnaris* organisms fed to juvenile coho salmon offer protection against the disease.

Discussion

Our studies suggest that oral immunization may be a practical and effective method for protecting juvenile salmonids against columnaris disease. Columnaris vaccine incorporated into fish food may be useful to state and federal hatcheries rearing salmonids, especially those hatcheries rearing fish in warm water. Fish reared in columnaris-free hatcheries may also benefit, since the immunized fish, in most instances, would eventually be released into the river and be exposed to the disease. If *C. columnaris* antibodies of sufficient titers can be developed in young salmonids under hatchery production conditions, the fish may be protected against the disease during seaward migration. If the salmon retain *C. columnaris* antibodies during their ocean life, immunity may develop in the adults through natural challenge during upstream migration.

The initial increase in *C. columnaris*-associated mortality of rainbow trout approximately 3 months old, followed by an equally aburpt decrease (Fig. 1) suggests that a natural protective mechanism was developed through exposure to *C. columnaris* in river water. Crowded conditions in the hatchery troughs were indirectly beneficial to the trout. Although *C. columnaris*-associated mortalities were high, the weaker fish were eliminated from the population and most survivors probably developed a protective mechanism against the pathogen. Most yearling survivors were immune to a second annual infection of C. *columnaris*. The challenges probably increased antibody synthesis to levels sufficient to protect the fish. If a severe infection should initially occur before antibody levels are sufficiently high, the protective mechanism would probably be too low to protect the fish.

The presence of immune carrier fish capable of releasing large numbers of *C. columnaris* organisms into the water is probably the major source of salmonid infection in the Columbia River. Fish ladders with high population density of coarse fishes during migration of salmonids are the most likely sites of exposure and infection (Fujihara and Hungate 1971). Since nonexposed fish appear to be very susceptible to infection, the release of heavy concentrations of the pathogen with the correspondingly low water flow in ladders would create ideal conditions for infection. Perhaps a deliberate exposure of hatchery-reared salmonids to avirulent or low-virulence *C. columnaris* organisms may be helpful in inducing resistance in the fish.

Acknowledgments

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Segregation Between Adult Cutthroat Trout (*Salmo clarki*) and Dolly Varden (*Salvelinus malma*) in Small Coastal British Columbia Lakes[1]

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ANDRUSAK, H., AND T. G. NORTHCOTE. 1971. Segregation between adult cutthroat trout (*Salmo clarki*) and Dolly Varden (*Salvelinus malma*) in small coastal British Columbia lakes. J. Fish. Res. Bd. Canada 28: 1259–1268.

Comparison of spatial distributions and food of allopatric populations of cutthroat trout (*Salmo clarki*) and Dolly Varden (*Salvelinus malma*) in two lakes and of sympatric populations in a third lake suggested interactive segregation. Allopatric cutthroat changed their depth distribution throughout the spring and summer but no diel migration was noted. Changes in food habits closely corresponded to seasonal changes in vertical distribution. A wide range of food organisms was eaten from surface, midwater, and benthic sources. Allopatric Dolly Varden underwent diel changes in spatial distribution, with many being caught in onshore waters near the surface at night. They were absent from such areas in the day, apparently occupying offshore waters at greater depths. Their diet throughout summer consisted largely of surface insects and zooplankton, with lesser amounts of bottom fauna.

Sympatric cutthroat trout and Dolly Varden were spatially segregated throughout the summer. Cutthroat trout occurred and fed mostly near the surface in littoral areas. In contrast, Dolly Varden were offshore and benthic in distribution and fed primarily on bottom organisms. By comparing distribution and food habits in allopatric and sympatric populations, it was suggested that competition for food may contribute to their segregation in sympatry. Although segregation of these species when cohabiting may involve interactive processes, the possibility of selective differences being of importance cannot be dismissed.

ANDRUSAK, H., AND T. G. NORTHCOTE. 1971. Segregation between adult cutthroat trout (*Salmo clarki*) and Dolly Varden (*Salvelinus malma*) in small coastal British Columbia lakes. J. Fish. Res. Bd. Canada 28: 1259–1268.

Une comparaison de la distribution spatiale et de la nourriture de populations allopatriques de truites cutthroat (*Salmo clarki*) et d'ombles d'Orégon (*Salvelinus malma*) dans deux lacs et de populations sympatriques dans un troisième donne à entendre qu'il y a ségrégation à action réciproque. La répartition verticale des truites cutthroat allopatriques varie tout au cours du printemps et de l'été, mais aucune migration journalière n'a été observée. Les changements saisonniers de profondeur sont accompagnés de changements correspondants d'habitudes alimentaires. Il y a diversité dans la gamme des aliments attrapés en surface, dans la couche intermédiaire et sur le fond. La distribution spatiale de la population allopatrique d'ombles d'Orégon change au cours de la journée: plusieurs sont capturés en surface près du rivage la nuit. Ils délaissent ces régions pendant le jour, vraisemblement pour les eaux du large et de plus grande profondeur. Leur alimentation estivale consiste surtout en insectes et zooplancton de surface et, à un degré moindre, en faune benthique.

Les populations sympatriques de truites cutthroat et d'ombles d'Orégon occupent des territoires différents pendant tout l'été. Les truites cutthroat habitent surtout la zone littorale superficielle où elles se nourrissent. Par contre, les ombles d'Orégon se trouvent près du fond plus au large et se nourrissent surtout d'organismes benthiques. En comparant la répartition et le régime alimentaire des populations allopatriques et sympatriques, on est porté à croire que la compétition pour la nourriture contribue à les rendre sympatriques. Quoique la ségrégation, au moment où ces espèces occupent un même territoire, puisse impliquer des procédés d'action réciproque, il ne faut pas écarter la possibilité que les différences sélectives aient un rôle important.

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(FOOTNOTES 1, 2, 3 *next page*)

DOLLY Varden and cutthroat trout frequently occur together in many lakes of coastal British Columbia (Andrusak and Northcote 1970). With the exception of the brief account by Godfrey (1955) there has been no detailed study of their ecology and feeding habits in such lakes.

Nilsson (1955, 1960, 1961, 1963, 1965) has examined competitive interaction between arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) and has developed the hypothesis of "interactive segregation," originally used by Brian (1956) in his work on the ant genus *Myrmica*. According to Nilsson (1967) the term means "that the ecological differences between species (eg. in food or habitat selection) are often magnified through interaction, i.e. the species segregate into different niches through competition and/or predation." Brian (1956) also describes a second type of segregation termed selective segregation, which "consists of the selection by instinctive behaviour of totally different habitats (that is resources and modifying factors combined)." On the basis of nearly 20 years of data, Nilsson (1965, 1967) hypothesizes that food segregation in fish is interactive and due to one or more mechanisms such as food competition (exploitation and interference), territoriality, food fighting, and predation. Clearly then, interactive segregation is not a description of the mechanisms involved in population interactions, but rather it describes the results of these mechanisms.

The hypothesis of interactive segregation has been supported to some extent by experimental and field observations of Hartman (1965) who noted spatial segregation between underyearling coho salmon and steelhead trout during the spring and summer in laboratory experiments as well as in small coastal streams of British Columbia. Nilsson (1967) cites several other instances of interactive segregation including that of Gee and Northcote (1963) on food segregation between two closely related species of dace (*Rhinichthys*) in the Fraser River system. Although several workers have described interactive segregation between coexisting fish species based on extensive food analysis, few have attempted to relate food habits and distribution with the phenomenon.

The present field study conducted during 1967 investigated the possibility of segregation between sympatric populations of adult coastal cutthroat trout and Dolly Varden in Marion Lake, compared these with allopatric populations of each species in two lakes, and examined relations between them in the light of Nilsson's hypothesis of interactive segregation.

Description of Study Areas

Three lakes in the lower Fraser Valley of southwestern British Columbia were studied (Fig. 1); all lie in the Coast and Insular Mountain limnological region (Northcote and Larkin 1956).

Placid Lake (elevation 503 m) is shallow with a mean depth of 4.3 m and a maximum of 6.7 m. The lake becomes thermally stratified early in the summer and later shows low oxygen near the bottom (Fig. 2). Placid Lake contains cutthroat trout, the only species of fish present.

Dickson Lake (elevation 671 m) is deep and steep-sided with a mean depth of 35.6 m and maximum of 76 m. A thermocline develops by midsummer between 3 and 6 m, but there is no evidence of oxygen deficiency (Fig. 2). For comparison with Marion and Placid lakes, a shallow bay near the outlet was used as the study area. Dickson Lake contains Dolly Varden, the only species of fish present.

Marion Lake (elevation 533 m) is shallow with a mean depth of 4.5 m and maximum of 12.1 m. Only slight thermal stratification is evident during late summer, although oxygen is low in the deeper regions in September (Fig. 2). The lake contains cutthroat trout and Dolly Varden, neither of which at maturity exceed 25 cm in length. No other species of fish are present.

Methods

Temperature, oxygen, and Secchi disc readings were taken with standard techniques at stations (Fig. 1) on each of the lakes during the study. To record vertical distribution of fish in spring and summer of 1967, gillnets were fished in Marion Lake at weekly intervals. Nets were set at monthly intervals in Placid Lake and twice (June and July) in Dickson Lake. In autumn, single nettings were made in Placid and Dickson lakes and two in Marion Lake. Two central and two littoral stations were established at Marion Lake; these appeared adequate to describe distributions of adult fish in the small lake, as did those established for the even smaller Placid Lake (Fig. 1). Three netting stations were located in shallow bays of the larger Dickson Lake and were intended to sample the same depth intervals as on the other lakes, but of course could not reveal fish distribution in the whole lake. In all instances, monofilament (0.2 mm) nylon nets were used with stretched mesh sizes of 25, 38, and 51 mm and depths of either 3, 5, or 10 m. Only adult or near-adult fish were caught (Placid Lake cutthroat 113–253 cm length; Dickson Lake Dolly Varden 120–226 cm; Marion Lake cutthroat 108–223 cm,

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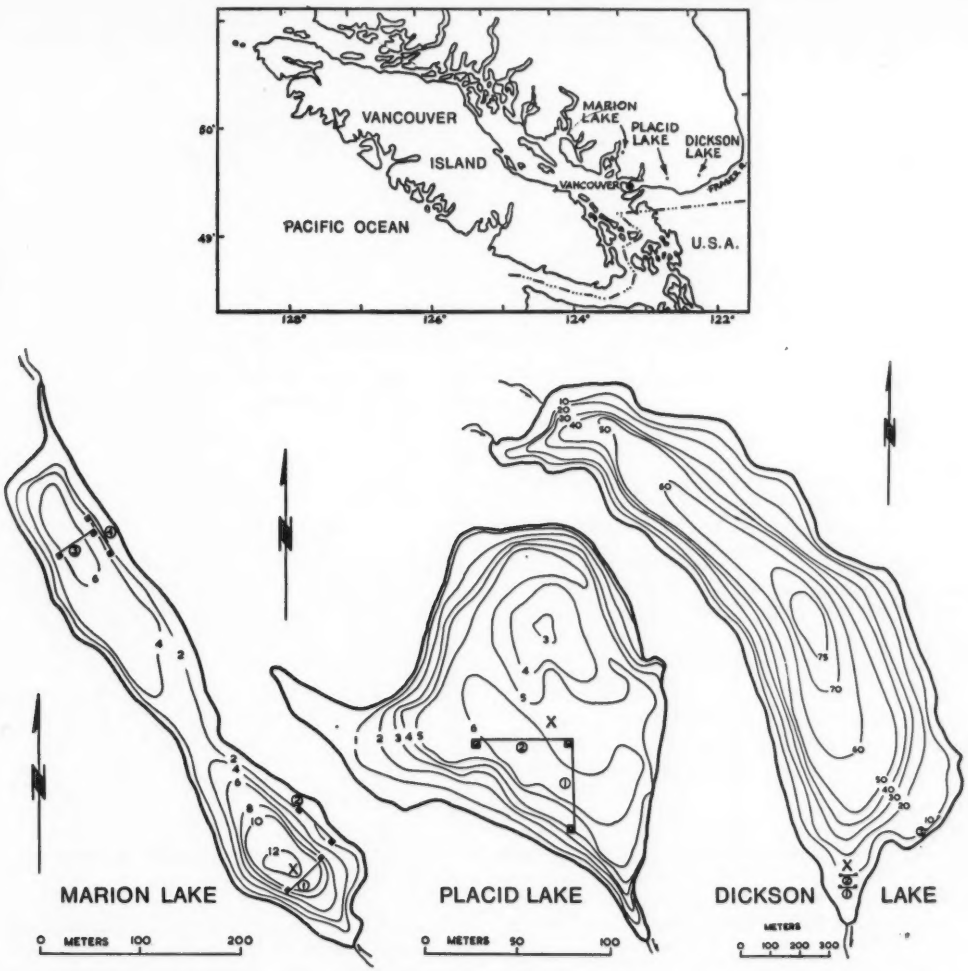

FIG. 1. Location and morphometry of three coastal British Columbia lakes. Depth contours in meters; X indicates limnological station; ■—■ indicates netting station.

Dolly Varden 100–212 cm). Nets usually fished the total water column from surface to bottom at each locality. Each net consisted of three sections (each 5 m in length) with one mesh size per section. Normally, a gang of three nets was used for each series, so that 45 m of net were fished per station. Nets were marked horizontally at 1-m intervals for convenience in recording fish distribution. Nets were fished in all lakes from midafternoon to dusk for a minimum of 1.5 hr and usually for 3 hr. Overnight sets were made to obtain information on nocturnal distribution of fish. Netted fish were preserved in 10% formalin shortly after collection.

Chi-square tests (Siegel 1956) were used to determine if significant differences ($P = 0.05$) existed in vertical and horizontal distributions of Marion Lake fish and in proportion of catches of both species between day and night sets. Several methods of stomach analysis were tested (volume displacement, number, and percent occurrence) and, as only small variations were evident between the results of each method, only one (percentage of total volume) was presented.

Further details on methodology and statistical analysis are given in Andrusak (MS 1968).

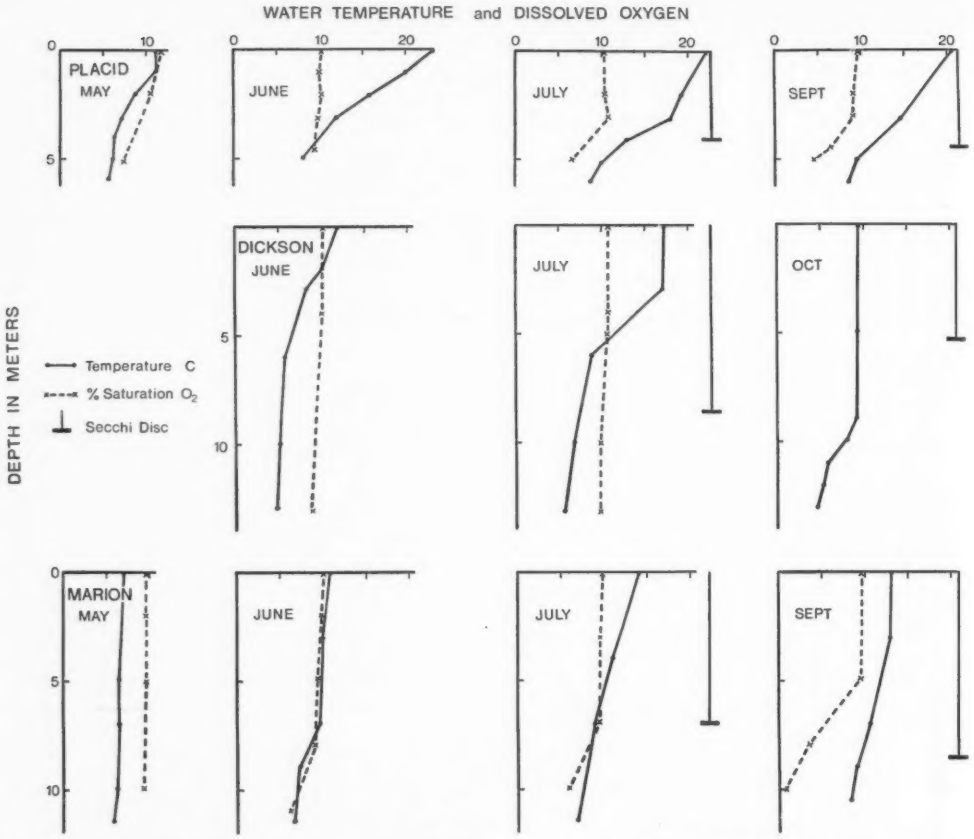

FIG. 2. Temperature, oxygen saturation (multiply scale by 10 for percent), and light penetration at limnological stations (see Fig. 1) in the three study lakes during spring to autumn 1967.

Fish Distribution

During early spring, most cutthroat in Placid Lake were caught in near-surface waters (Fig. 3). As summer progressed, fish apparently moved to deeper water, particularly between 4 and 5 m. No appreciable difference in vertical distribution was noted between day and night. In late fall prior to ice formation the few cutthroat taken were near the bottom. No onshore concentrations of cutthroat were noted in Placid Lake nor were there significant differences horizontally between their day and night distribution.

Virtually no Dolly Varden were caught in nets set near shore (10 m or less) during the day at Dickson Lake (stations 1–2). Echo-sounding traces (not shown) indicated that in daytime the fish were at considerable depths (about 25 m) but during darkness moved towards the surface and onshore. Many were caught in the near-shore nets at night (Fig. 4), particularly during summer when they were widely distributed within the upper 10 m.

In early spring, vertical night distributions of cutthroat and Dolly Varden in Marion Lake were not significantly different (Fig. 5). No fish were caught in day sets. Both species were concentrated near the bottom, the Dolly Varden markedly so.

Distribution of both species during the summer (Fig. 5, 6) were quite different and clearly demonstrated spatial segregation, particularly at the deep, central station (Fig. 6). Most cutthroat were caught in the upper 3 m in summer months and most Dolly Varden below 5 m. A similar distribution pattern (not shown) was found in Phyllis Lake (adjacent to Marion Lake). The proportions of both species

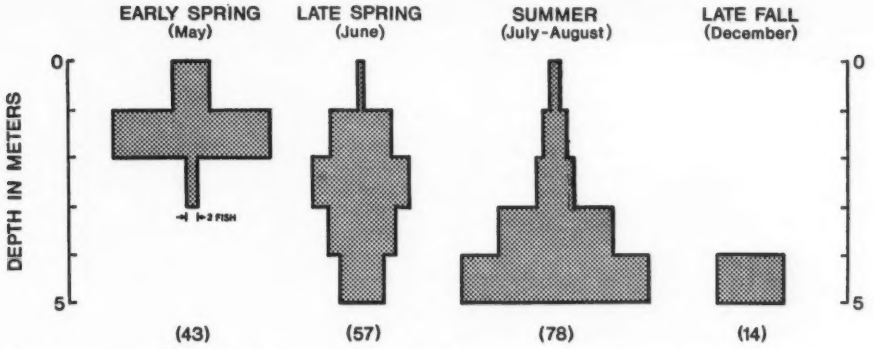

FIG. 3. Vertical distribution of allopatric cutthroat trout in 5-m deep gillnet sets at stations 1 and 2 at Placid Lake, 1967; sample size in parentheses.

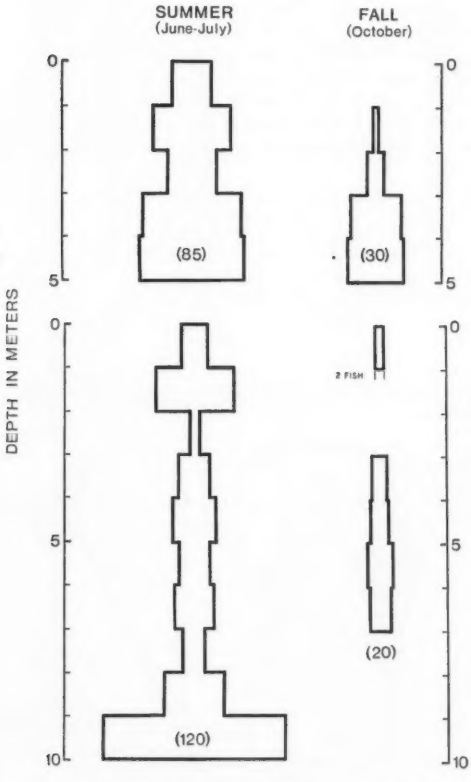

FIG. 4. Vertical distribution of allopatric Dolly Varden in 5- and 10-m deep gillnet sets at stations 1, 2, and 3 at Dickson Lake, 1967; sample size in parentheses.

caught at night in shallow (0–3 m) and deeper (4–10 m) intervals of the 10-m nets did not differ significantly from those caught in the day; consequently, day and night data were pooled.

Although spatial segregation between the two species was still evident in late summer, concentration of Dolly Varden in the deep water was not apparent (Fig. 6). Cutthroat distribution remained relatively unchanged.

In fall, cutthroat trout commonly were taken at night below 5 m so that vertical distribution of the two species was similar (Fig. 6). No fish were caught during the day.

Twice as many cutthroat trout as Dolly Varden were caught during summer in 3-m nets set parallel to shore (station 2; data not shown). This suggested a horizontal difference in distribution, substantiated in data from the 10-m net sets (Fig. 7). Significantly more cutthroat trout were caught in the onshore than in the middle or offshore nets. No significant differences were found in numbers of Dolly Varden taken in the onshore, middle, or offshore nets, but their marked concentration near the bottom in the onshore net was not evident in the offshore net.

Throughout the summer, Dolly Varden were caught in clusters in the nets, whereas cutthroat were usually isolated or rarely in groups of two or three fish. Statistical analysis of the distribution patterns recorded from the netting data showed that Dolly Varden in 10-m nets were highly contagious, whereas cutthroat were dispersed in 10-m nets and randomly distributed in 3- and 5-m nets (see Andrusak MS 1968 for details).

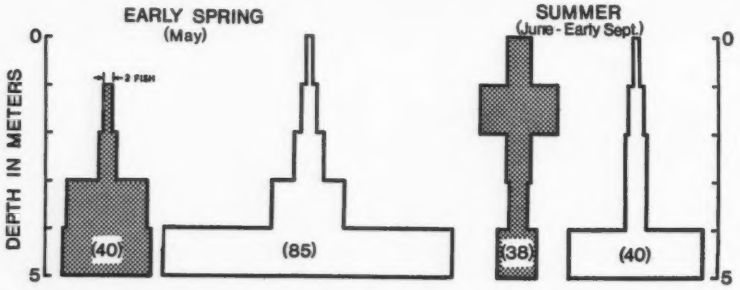

FIG. 5. Vertical distribution of sympatric cutthroat trout (stippled) and Dolly Varden (blank) in 5-m deep gillnet sets at stations 3 and 4 at Marion Lake, 1967; sample size in parentheses.

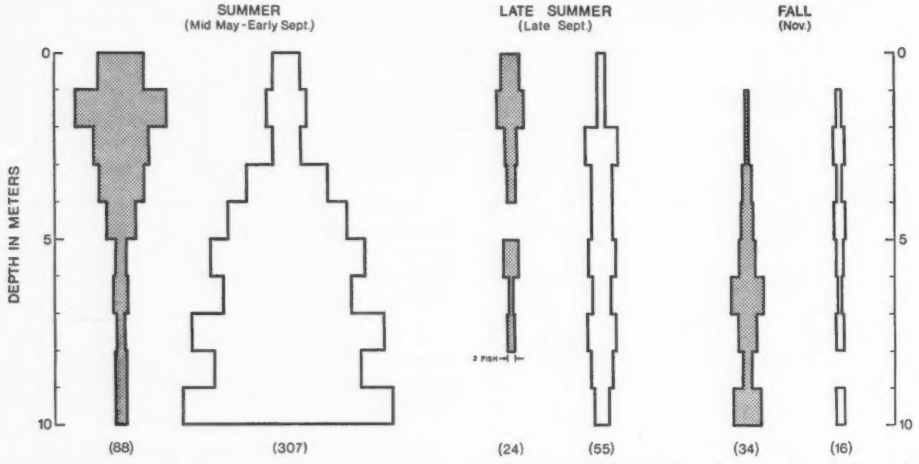

FIG. 6. Vertical distribution of sympatric cutthroat trout (stippled) and Dolly Varden (blank) in 10-m deep gillnet sets at station 1 at Marion Lake, 1967; sample size in parentheses.

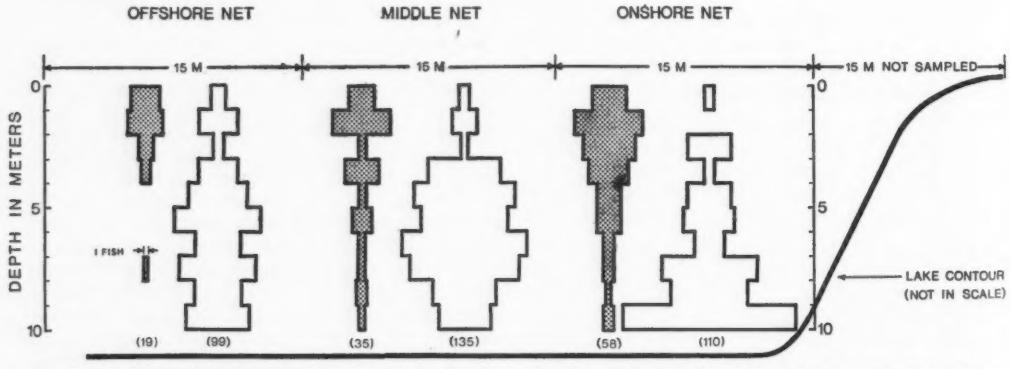

FIG. 7. Vertical and horizontal distribution of sympatric cutthroat trout (stippled) and Dolly Varden (blank) in 15-m lengths of 10-m deep nets set at station 1 at Marion Lake during summer (June–early September) 1967; sample size in parentheses, ca. 1 m at bottom not sampled.

Food Habits

Marked seasonal changes were evident in food habits of cutthroat trout in Placid Lake (Fig. 8). In spring, chironomid pupae were the most important food, but as summer progressed, zooplankton (largely *Diaptomus* spp.) and then bottom fauna (especially trichopteran larvae) became dominant items and retained this prominence into fall. The seasonal change in diet from planktonic to benthic forms coincided with the shift in vertical distribution of the fish (Fig. 3). Although cutthroat in Placid Lake apparently did not feed intensively on surface material, they nevertheless did utilize a considerable variety of food from most areas of the lake (surface, midwater, and bottom) over the feeding season.

Dolly Varden in Dickson Lake fed heavily on terrestrial insects during summer (Fig. 8). Because these fish were caught only in the evening or night, results do not necessarily reflect food habits over a 24-hr period. However, if they had consumed appreciable amounts of zooplankton or bottom fauna during the day, one would expect these items to be represented in greater quantities in fish caught after dusk. In the fall, zooplankton was the dominant food, with terrestrial insects and bottom fauna of secondary importance. These Dolly Varden drew heavily upon surface organisms over the feeding season but also utilized planktonic and benthic forms to a considerable degree.

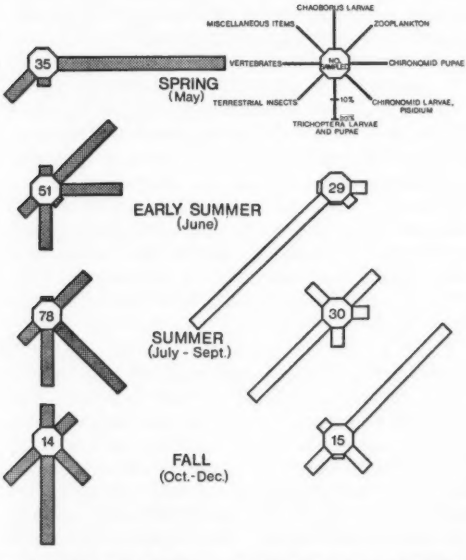

FIG. 8. Stomach contents (average percent volume) of allopatric Placid Lake cutthroat (stippled) and Dickson Lake Dolly Varden (blank). 1967.

Cutthroat trout and Dolly Varden in Marion Lake had consistently different diets during late spring and summer of 1967 (Fig. 9). Dolly Varden fed heavily on chironomid pupae in early spring but later became primarily bottom feeders. Their diet consisted mainly of chironomid larvae, *Pisidium*, and to a lesser extent trichopteran larvae. With the exception of June, terrestrial insects played a very minor role in the diet of these Dolly Varden. During fall, trichopteran and chironomid larvae were of considerable importance.

In contrast to the bottom feeding habits of Dolly Varden in Marion Lake, cutthroat were heavily dependent upon terrestrial insects after early spring. In late spring, nearly 80% of their diet was composed of adult beetles such as *Zylodromus concinnus*, *Pelecomalium testaceum*, and *Podabrus diversipes*. Increased numbers of dipteran adults (*Chironomidae*, *Simulidae*, *Culicidae*, and *Empididae*) were represented in the late summer samples. Trichopteran larvae (*Mystacides alafimbriata* and *Ptilostomis* sp.) were of secondary importance to cutthroat in spring and summer but formed 61% of their diet (chiefly *Glyphosyche irrorata*) in the fall. Typical benthic organisms such as oligochaetes, *Pisidium*, and chironomid larvae never exceeded 2% of the average volume of the cutthroat diet during 1967.

Zooplankton was not abundant in Marion Lake (data not shown) until mid-July and did not contribute appreciably to the diet of either species until late summer. In general, Dolly Varden preyed upon cladocerans (*Daphnia rosea* and *Holopedium gibberum*) whereas cutthroat fed largely on calanoid copepods (*Diaptomus leptopus*).

Discussion

That there were differences in the spatial distribution of the allopatric populations of cutthroat trout and Dolly Varden is perhaps not surprising considering dissimilarities of the two lakes studied. Placid Lake, a small, shallow, boglike body of water, showed sharp vertical temperature changes by June with surface temperatures over 20 C. At the same time surface waters in the much larger and deeper Dickson Lake had barely exceeded 10 C. Cutthroat trout in Placid Lake did not appear to undergo diel migrations (either vertically or horizontally) but did change their distribution seasonally, probably in response to high temperatures in the upper layers. During early spring they occurred largely in near-surface waters but later were most abundant

in deeper parts of the lake. Dolly Varden in Dickson Lake, although not exhibiting any marked seasonal changes in distribution, apparently underwent a vertical and horizontal migration, moving from midwater offshore regions in the day into shallow onshore areas at night. In summer, they did not appear to be restricted to deeper regions of the lake and commonly were taken in the upper 5 m.

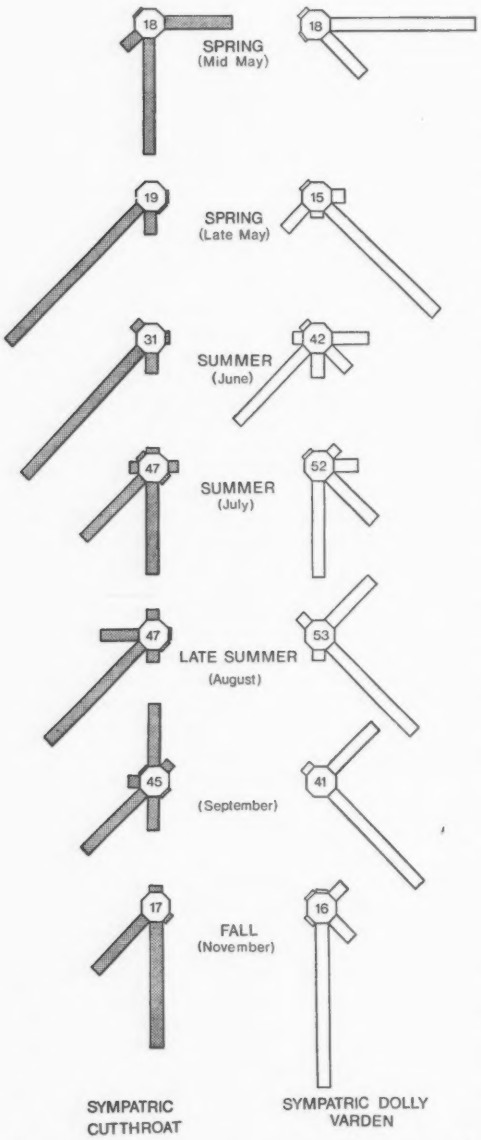

FIG. 9. Stomach contents (average percent volume) of sympatric Marion Lake cutthroat (stippled) and Dolly Varden (blank), 1967; see Fig. 8 for legend.

The two species coexisting in Marion Lake showed striking differences in spatial distribution, at least during summer. Cutthroat trout then remained near the surface and shore, whereas Dolly Varden inhabited deeper water. Similar divergences between these two species have been observed in Marion Lake (Schutz and Northcote 1972) and in other coastal lakes (Andrusak and Northcote 1970).

Spatial segregation has been noted by Nilsson (1955, 1961) between brown trout and arctic char coexisting in some Swedish lakes and Everhart and Waters (1965) have recorded a similar phenomenon for arctic char and brook trout in Maine lakes. Hartman (1965) demonstrated that spring and summer microhabitat segregation of juvenile coho salmon and steelhead trout broke down during fall and winter. Nilsson (1967) points out that Hartman's field and experimental evidence suggests that interactive segregation may be seasonal in occurrence. Seasonal changes in vertical distribution of cutthroat and Dolly Varden in Marion Lake support this view. In spring, vertical distributions of the two species were similar, both being found together in deeper areas of the lake. As summer progressed, spatial segregation became quite distinct. By late fall, vertical distribution of cutthroat changed, most fish being caught at depths greater than 5 m. Indeed, their distribution then appeared more similar to that in early spring rather than summer.

Thus, spatial segregation between sympatric populations of the two species was clearly evident during the summer, cutthroat occupying surface onshore waters and Dolly Varden deeper near-bottom layers. In contrast where the species lived allopatrically, each appeared to utilize more completely all available habitat, in both shallow and deep water.

Food habits of allopatric cutthroat trout and Dolly Varden showed some dissimilarities. Bottom fauna formed an important item in the diet of cutthroat, particularly later in summer, whereas terrestrial insects were predominant in the Dolly Varden diet. However, zooplankton was a secondary but significant food source for both species. Differences in feeding may be due to differences between the lakes studied and to the differences in spatial distribution of the fish noted above. Nevertheless, over the feeding season, allopatric populations of each species did utilize a broad spectrum of food organisms, drawn from benthic, midwater, and surface zones. This is in agreement with observations

of Nilsson (1965) summarizing food habits of allopatric salmonids.

Insight into the process of segregation can be gained by analysing food habits of cohabiting species. Nilsson (1965) hypothesized that food habits of sympatric salmonid species would be dissimilar. Differences in food habits of the two species of fish in Marion Lake during summer adds further support to this hypothesis. Cutthroat fed primarily on surface foods (largely terrestrial insects), whereas Dolly Varden concentrated on bottom fauna, especially chironomid larvae and *Pisidium*. In contrast to the allopatric populations, the sympatric populations in Marion Lake each utilized a different and narrow range of food items during summer. Food habits apparently reflected the respective differences in spatial distribution of the two species. Field and experimental observations of Schutz and Northcote (1972) on feeding behaviour of Marion Lake cutthroat and Dolly Varden were in general agreement with these results.

Nilsson (1960) has presented evidence that food segregation in arctic char and brown trout became progressively more pronounced as summer advanced. Gee and Northcote (1963) suggest a similar phenomenon with leopard dace and longnose dace in the lower Fraser River. Likewise, in the present study, food segregation was most distinct in late summer when sympatric Dolly Varden concentrated on chironomid larvae and *Daphnia rosea*, whereas sympatric cutthroat fed chiefly on terrestrial insects and *Diaptomus leptopus*.

The relatively high amounts of terrestrial insects taken both by Dolly Varden and by cutthroat in June can possibly be explained by what Nilsson (1965), Keast (1965), and others refer to as "superabundance" or "mass development" of a food type. They suggest that when a food is in obvious superabundance, feeding habits of fish species will be similar — a situation that may well occur when terrestrial insects fall onto lake surfaces in great numbers during the summer (Norlin 1967). Coleopteran and hymenopteran adults were observed in considerable numbers on the surface of Marion Lake during June and early July.

In late autumn, food segregation was less pronounced than during summer. Both species consumed trichopteran larvae in sizeable quantities and, although this also could have been a temporary response to a superabundance of these larvae, the marked changes in diet suggest that segregation was largely a summer phenomenon. The switch to bottom-dwelling organisms also indicates a change towards winter feeding conditions, since the quantities of zooplankton (Andrusak MS 1968) and terrestrial insects had diminished by late fall. The latter in any event became unavailable with formation of ice cover by December.

Interspecific competition for food has been described by Larkin (1956) as "the demand, typically at the same time, of more than one organism for the same resources of the environment in excess of immediate supply." But as Northcote (1954) and others have pointed out, it is extremely difficult to measure whether the demand is greater than the supply. As a result, it is rarely possible to demonstrate directly from field work that food competition occurs between fish species. Results from this study were no exception and at best only inferences can be made concerning food competition between the two species of fish in Marion Lake. Spatial and food segregation were clearly demonstrated to be seasonal in occurrence; hence, some explanation is required to indicate how such segregation may become reestablished every summer. Perhaps in early spring, changes in both distribution and diet of cutthroat trout are the result of exploitation (i.e., utilization of resources by one species more effectively than by the other), one of the mechanisms that Nilsson (1965) suggests is functional in interactive segregation. Schutz and Northcote (1972) indicate in feeding experiments that cutthroat trout exploit the surface food resources much more quickly than do Dolly Varden. Hartman (1965) has demonstrated experimentally that seasonal differences in space requirements of underyearling coho and steelhead are due to high levels of aggression during spring and summer.

Nilsson (1967), in reviewing the concept of interactive segregation, suggests the mechanisms that may operate in this process are territoriality, exploitation, food fighting, predation, and other interference. The dispersed and random distributions of netted Marion Lake cutthroat, in contrast to the clumped distribution of Dolly Varden, could be interpreted as indicating territoriality in the former species. Furthermore, the cutthroat appeared to be more clearly associated with the littoral regions of the lake where loosely organized feeding areas might be more readily established and defended. Certainly adult Marion Lake cutthroat show aggressive behaviour in the laboratory (Schutz and Northcote 1972) and Newman (MS 1960) suggests that juvenile cutthroat are territorial. Regarding predation, both species in the lake fed on fish, although only to a minor degree. Both species can be highly predacious in other coastal lakes (Andrusak and Northcote 1970).

During summer, allopatric populations of Dolly Varden and cutthroat utilized a broad spectrum of food organisms, in direct contrast to the sympatric populations of the same species in Marion

Lake. This suggests that these two salmonids may be quite flexible in their food habits and raises the question as to whether in the sympatric situation, observed restrictions of both spatial distribution and diet were solely a reflection of interactive segregation. It would seem doubtful that spatial and food differences demonstrated in Marion Lake were due entirely to interactive segregation or, on the other hand, to selective segregation, i.e., to innate differences in behaviour. Both processes may be involved.

Acknowledgments

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Investigations Into the Relation Between Integrated Echo Voltage and Fish Density[1]

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THORNE, R. E. 1971. Investigations into the relation between integrated echo voltage and fish density. J. Fish. Res. Bd. Canada 28: 1269–1273.

An echo integrator, working in combination with an echo sounder, measures the total voltage of the fish echoes received from a given depth interval and sums these voltages over time. Investigations were made on the relation between integrated echo voltage and fish density for juvenile sockeye salmon (*Oncorhynchus nerka*) in Lake Washington and Pacific hake (*Merluccius productus*) and Pacific herring (*Clupea harengus pallasi*) in Puget Sound. When fish were distributed at densities at which they could be acoustically resolved as individual targets, the relation between net catch and integrated voltage was linear. At higher densities when the fish echoes were multiple targets, the net catch was related to the square of the integrated voltage.

THORNE, R. E. 1971. Investigations into the relation between integrated echo voltage and fish density. J. Fish. Res. Bd. Canada 28: 1269–1273.

L'intégrateur d'échos, couplé à un sondeur à écho, mesure le voltage total des échos de poissons à des intervalles de profondeur donnés et intègre ces voltages sur le temps. Nous avons étudié la relation entre le voltage intégré des échos et la densité des espèces suivantes: le saumon sockeye juvénile (*Oncorhynchus nerka*) dans le lac Washington, le merlu (*Merluccius productus*) et le hareng du Pacifique (*Clupea harengus pallasi*) dans le détroit de Puget. Lorsque la densité du poisson est telle que les échos se résolvent en cibles individuelles, la relation prise-voltage intégré est rectiligne. A des densités plus fortes, alors que les échos représentent des cibles multiples, la prise est fonction du voltage intégré au carré.

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TRADITIONALLY, the magnitude of commercial fish populations has been estimated either directly from recoveries of marked members or indirectly from catch per unit of effort. These methods require substantial effort, expenditure, and historical data; the use of acoustic equipment is a promising alternative.

Several types of electronic apparatus for quantifying returning echo signals have been developed. These instruments are primarily various types of pulse counters (Mitson and Wood 1961; Carpenter 1967; Dowd 1969; Cushing 1968; Craig and Forbes 1968). An echo integrator (Dragesund and Olsen 1965) measures and sums the voltage amplitudes from fish echoes, rather than counts the number of echoes. Application of both counters and integrators has been mostly limited to situations in which the echo targets are primarily individual fish (Cushing 1968; Midttun and Nakken 1968).

When fish of the same kind and size are sparsely and evenly distributed over a narrow depth range so that there are no overlapping of echoes from individual fish, the output of both counters and integrators is directly proportional to the number of fish encountered (Thorne and Lahore 1969). When fish are more densely concentrated, echoes from two or more fish will be received at about the same time. A simple pulse counter cannot distinguish between single and multiple targets, thus the number of targets counted is an underestimate of the number of fish encountered. The cycle counter described by Carpenter (1967) can be set to distinguish between single and multiple targets in many instances, but is still limited to a counting rate of one fish per pulse length. The amplitude of a multiple target is theoretically related to the square root of the number of fish within the echo (Truskanov and Scherbino 1966; Lahore and Lytle 1969). At high densities the echo amplitude will also be

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affected by indirect scattering, sound absorption, and shading.

An echo integrator designed and built by H. W. Lahore at the University of Washington (Lahore MS 1969) was utilized to investigate the relation between integrated echo voltage and fish density. Investigation was made on stocks of three species of fish, juvenile sockeye salmon (*Oncorhynchus nerka*), Pacific hake (*Merluccius productus*), and Pacific herring (*Clupea harengus pallasi*), and density was estimated with midwater trawls.

Materials and Methods

The Echo Integrator

An electronic integrator is a device that has at its output terminal the integral over time of the voltage that has been applied to its input. A block diagram of the basic integrator system is shown in Fig. 1. The time base is triggered by a pulse from the echo sounder. Using this time base, the depth control circuit turns on the relay for the depth interval to which it has been set. Three integrator models were used during this investigation. In the initial echo integrator system the signal from the echo sounder was taken from the stylus output. There was no time-varied-gain (TVG) circuit in this design. The system was modified and a TVG circuit, which amplified the signal voltage in proportion to depth (20 log R in decibels, where R = depth), was installed in the second model to correct for one-way spreading loss. No correction for absorption was included since this term is minor at the frequency and depth interval involved in this study.

The TVG circuit was optional in the third model. Theoretically, a depth correction of 20 log R in decibels (db) is necessary for multiple targets. However, no TVG is required for exclusively single fish targets, since the volume insonified by the echo sounder pulse at depth, R, termed the *pulse resolution volume*, increased in proportion to R^2, the same rate as the individual fish echo strength decreases (Thorne MS 1970).

In the second and third integrator models, the signal was taken as an AC input from the IF part of the echo sounder, rectified, amplified, and put through the relay contacts to the integrator, peak detector, and pulse counter. The peak detector indicated the maximum amplitude of the signal in the gated depth interval. This indication allowed a relative comparison of the magnitude of signals from individual fish of different sizes or from schools of different densities. The pulse counter indicated the number of pulses in the gated interval that were over a certain amplitude. For low densities, this served to count the number of fish that had been in the examined depth interval. A more detailed description of the echo integrator and its associated electronics is given by Lahore (MS 1969) and by Lahore and Lytle (1970).

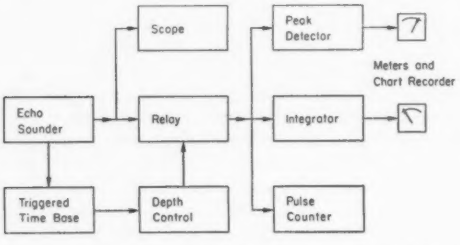


FIG. 1. Block diagram of the echo integrator system.

Field Procedures

The relation between integrated echo voltage and density of fish was investigated during eight cruises on Puget Sound from September 1968 to April 1969, and during six cruises on Lake Washington between September 1968 and July 1970. Investigations in both Lake Washington and Puget Sound were carried out from the University of Washington's research vessel *Commando*.

In cruises on Lake Washington in November 1969 and July 1970, a Simrad partner sounder with a frequency of 38.2 kHz was used. A 1.2-msec pulse length and a 10 × 32-cm transducer producing a beam with 20 × 7° angles between 3 db points was used in November, and a 0.4- msec pulse length and a 10 × 10-cm transducer producing 20° beam angles was used in July. All other data were obtained by the use of a Simrad EH2E sounder with a frequency of 38.2 kHz, a pulse length of 0.5 msec, and the 10 × 32-cm transducer. The first integrator model was used from September 1968 to January 1969, the second model from February to April 1969, and the third model in November 1969 and July 1970.

Net hauls in Lake Washington were made with a 10-ft Isaacs-Kidd midwater trawl (Isaacs and Kidd 1953) and at night. The forward sections of the net were constructed of 1½-inch stretch mesh. The codend was constructed with 1¼-inch stretch mesh and was lined with ½-inch stretch mesh. Net hauls were generally 10 min at a speed of 2.5 m/sec. In a 10-min haul the net swept approximately 10,000 m³. Net position was estimated from the length of towing wire and checked subsequently against bathykimograph (Marine Advisors, Inc., Model T-1c) records. Data from the net hauls were excluded when net position differed from estimated position by more than 4 m.

Net hauls in Puget Sound were made with a pelagic trawl (Marinovich Inc., Biloxi, Miss.). The net was 30 ft square in the mouth by 90 ft in length. It was constructed of 3-inch stretch mesh in the dogears, 2½-inch stretch mesh in the belly, 2-inch stretch mesh in the body, and 1½-inch stretch mesh in the funnel, and was equipped with a linear of ½-inch stretch mesh in the codend. Trawl duration varied from 5 to 30 min, but was generally 10 min. Speed of trawling was between 1.5 and 2.0 m/sec. Vertical and horizontal net openings while trawling were 5 m and 6 m, respectively. In a 10-min tow the net swept approximately 30,000 m³. Net position was determined by a pressure-sensitive electrical depth-

telemetry system. When telemetry was nonfunctional, depth was estimated from the amount of towing wire and was checked against bathykimograph records. Data from net hauls on Puget Sound were excluded when net position differed from estimated position by more than 7 m.

Integration was made simultaneously with net hauls over a 4–10 m depth interval centered on the estimated mean depth of the net. Integrator output was normalized by the time interval of sounding and the thickness of the depth interval, so that the final output was volts/600 sec × m), termed the *integration rate per meter*. The echo sounder–integrator systems were uncalibrated, so that values are strictly relative. Values obtained by use of the same system are generally comparable over a period of several months, but integration values from different systems are not directly comparable.

Results

Sockeye Salmon

A sample echogram and integrator output from Lake Washington are shown in Fig. 2. The juvenile sockeye salmon preponderate among the pelagic fish fauna of Lake Washington. The only other relatively abundant fish is the longfin smelt (*Spirinchus thaleichthys*). The net catches were subdivided into three size groups. The medium-size fish, ranging from 6 to 15 cm in length, included the juvenile sockeye salmon and adult smelt. Large fish, over 16 cm in length, included overyearling sockeye salmon, squawfish, and peamouth chub. Small fish, less than 6 cm, included sticklebacks, cottids, and juvenile smelt. Effects of size groups on the integrated voltage were analyzed by the use of multiple linear regression models. Because of its preponderance, only the medium-size group was significant in almost all instances (Thorne and Woodey 1970).

The numbers of juvenile sockeye salmon and adult smelt per 10-min tow are plotted against integration rates/meter for the six cruises in Fig. 3–5. Integration values are uniformly amplified by depth (no TVG), but effects of depth were minimal because of the relatively small depth range of the net hauls. Integration and net catch from the first five cruises appear to be linearly related. Correlation coefficients for the linear regression model,

$$N = bI, \qquad \text{Model 1}$$

where N equals the catch of juvenile sockeye salmon and adult smelt per 10-min tow, and I equals the integration rate/meter, were greater than 0.93 for the data from the first four cruises (Fig. 3 and 4). The highest net catch during these four cruises was 182 fish in a 10-min tow. Since the net swept approximately 10,000 m^3 in 10 min, the highest average density encountered was one fish per 55 m^3, on the assumption that net efficiency was 100%. The echo sounder (EH2E) could resolve individual targets differing more than 37.5 cm in distance from the transducer. Since the cross-sectional area of the cone at the depth of highest density of fish (23 m) was approximately 10 m^2, the pulse resolution volume at this depth was less than 4 m^3. Therefore, unless net efficiency was less than 7%, even the highest densities encountered during these four cruises were below an average of one fish per pulse resolution volume, and probably almost all of the echoes represented individual fish targets.

In cruises in November 1969 and July 1970, the Simrad partner sounder was used. In November, the model 1 linear correlation coefficient was 0.976, and maximum catch was 120 fish in 10 min. Fish densities in July were much higher; maximum catch was 1588 fish in 10 min. The relation between net catch and integration clearly deviates from linearity at the higher densities. A regression model of the form,

$$\log N = a + b \log I \qquad \text{Model 2}$$

was calculated for the six highest integration values. The resulting value of b was 2.02 with var $b = 0.024$, indicating density approximately related to (voltage)^2.

Pacific Herring

An echogram and integrator output from a haul taken through a layer of herring at night in Puget Sound are shown in Fig. 6. Catches per 10-min tow are plotted against integration rates/meter for day and night hauls containing at least 85% herring in Fig. 7. Integration values were uniformly amplified with depth. Catches at night appear to have increased linearly with integration, but variability associated with the net catch definitely increased with increasing integration.

The data from the hauls at night were analyzed by the model 2 regression equation. The log–log transformation normalizes the variance associated with the catch, and allows examination of the constant b. The data are replotted on log–log scales in Fig. 8. The value of b was calculated to be equal to 1.09, with a variance of 0.01. This value of b is not significantly different from a value of 1.0; thus the relation between net catch and integration is approximately linear for the range of densities encountered at night. The average depth of the net hauls was 14.5 m. At this depth a catch of 15,000 fish in a 10-min tow was equal

to a density of one fish per pulse resolution volume at 100% net efficiency. Catches in 18 of the 23 hauls were less than 3000 fish, and both the echogram and peak detector outputs indicated a nearly uniform, highly dispersed distribution at night.

Catches of herring during the day also appear to have been linearly related to integration. However, the slope of the regression line through the data is about six times that for the night data. During the daytime the fish were usually clustered in isolated schools, whereas at night they were more uniformly dispersed. Differences in both the catches and the integration rates calculated for the various hauls appear to be primarily differences in number and size of schools encountered during a haul, rather than differences in density of fish within schools. Estimated densities within schools averaged about 0.5 fish/m^3 on the assumption of 100% net efficiency. For virtually all schools, estimated densities were greater than an average of one fish per pulse resolution volume, and, correspondingly, ratio of catch to integration was high.

Pacific Hake

Catch and integration data on hake were obtained during three cruises in February, March, and April 1969. Hauls were made in Port Susan, a long narrow bay in the northern part of Puget Sound. Each winter large quantities of hake enter Port Susan from adjacent waters to spawn. They concentrate in the deeper water, generally between 5 and 10 m above the bottom, and occur rarely above 50 m. From February through April, the hake comprise about 95% by weight of all fish in the portion of the water column below 50 m. Lengths of fish are primarily between 32 and 41 cm and average 36 cm.

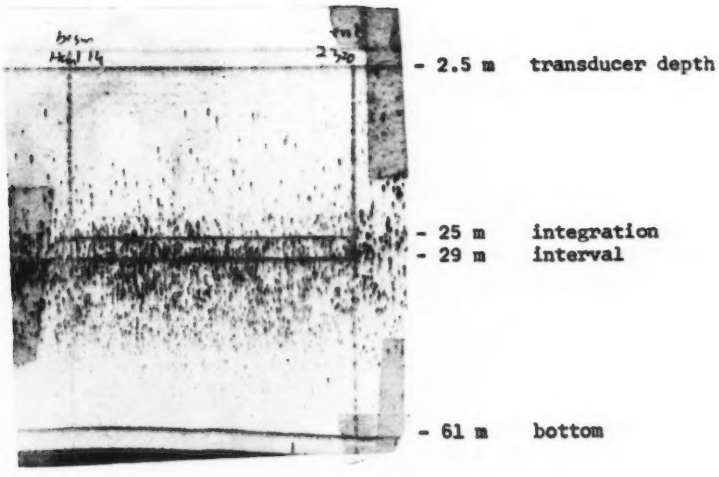
Catches per 10-min tow are plotted against integration rates/meter in Fig. 9. The integrated voltage is amplified in proportion to the depth (20 log R dB). The gating level in this system (integrator model 2) was insufficient to eliminate all low-level noise. The noise level was determined by measurement of the rate of integration when no fish marks were apparent on the echogram and was estimated to be 5.5. The relation between integration and catch was clearly not linear. Average depth of the hauls was 92 m. At this depth a catch of 240 lb is equivalent to an average density of one fish per pulse resolution volume, at 100% net efficiency. All seven of the trawls with integration rates/meter of 25 or more had catches averaging over one fish per pulse resolution volume. The model 2 regression equation was fitted to the catch and noise-corrected integration data from these seven high integration hauls. The resulting value of b was 1.95 with var b = 0.021. The value of b was not significantly different from 2.0.

Discussion

A linear relation between acoustic output and fish density is highly desirable for population estimation (Thorne MS 1970). Deviation of the relation between integrated echo voltage and fish density from linearity results from the fact that the echo sounder responds to acoustic pressure rather than intensity. If effects of indirect scattering, sound absorption, and shading are excluded, the average intensity is proportional to the number of fish. Since acoustic pressure is proportional to the square root of the average intensity, it follows that the voltage of an echo is proportional to the square root of the average number of fish within the pulse. Thus, a linear relation between integrated voltage and overall density can result only when the number of fish per echo is essentially constant, as the case is with single fish targets. On the assumption that net efficiency was independent of density, the results of the field studies indicate that when fish are fairly uniformly distributed, the relation between fish density and integrated echo voltage is approximately linear to densities approaching one fish per pulse resolution volume. When the fish density is even, the relation between fish density and integrated voltage will be linear to a density of exactly one per pulse resolution volume, and related to the square of voltage at higher densities. Realistically, we should expect the linear relation to become gradually exponential, depending on the amount of variability in the fish distributions.

Comparison of the results on herring between day and night show that a linear relation may exist between overall density and integrated voltage even though the echo pulses represent almost exclusively multiple fish targets. The apparent explanation is that the density of fish within schools is reasonably constant and independent of school size. The overall density and the integrated voltage thus depend primarily on the number and size of schools encountered, rather than on the density of fish within schools. A similar result was found for schools of cod during the day (Dowd et al. 1970). Population estimation with acoustic equipment may be greatly simplified if fish of a given species typically schooled at a fixed density.

Problems associated with multiple targets could be largely solved by installation of an analog squaring device in the integrator to square all the echo pulses. Integrator output would be proportional then to acoustic intensity. Further, if inte-



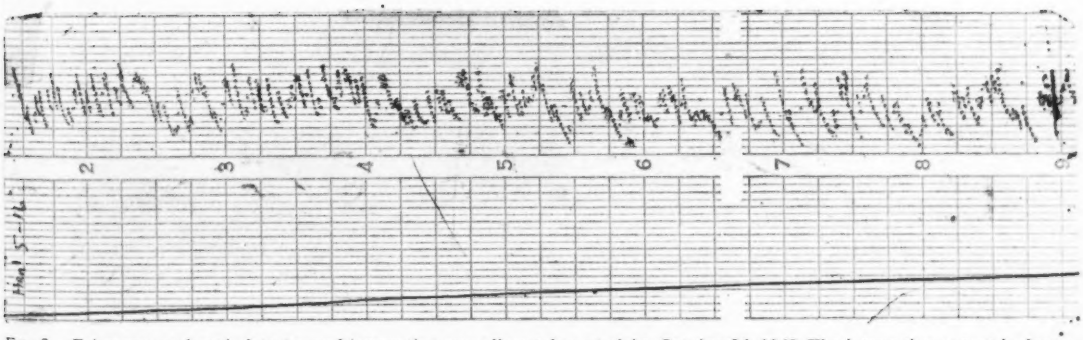


FIG. 2. Echogram and peak detector and integration recordings taken at night, October 26, 1968. The integration output is shown on the lower channel of the Rustrak chart paper; the peak detector output is recorded on the upper channel.

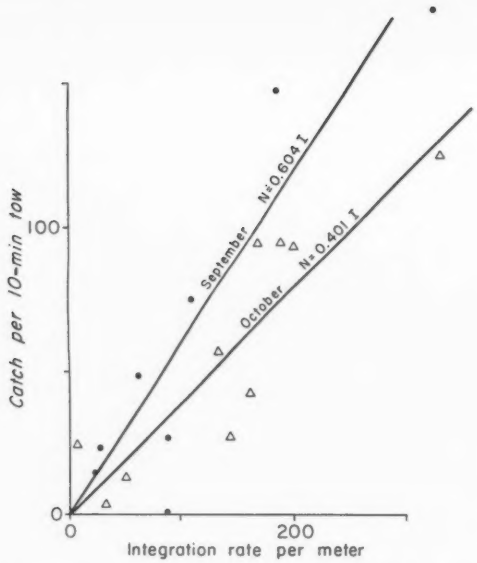


FIG. 3. Relation between numbers of juvenile sockeye salmon and adult smelt per 10-min tow and integration rates/meter taken at night, September (●) and October (△) 1968.

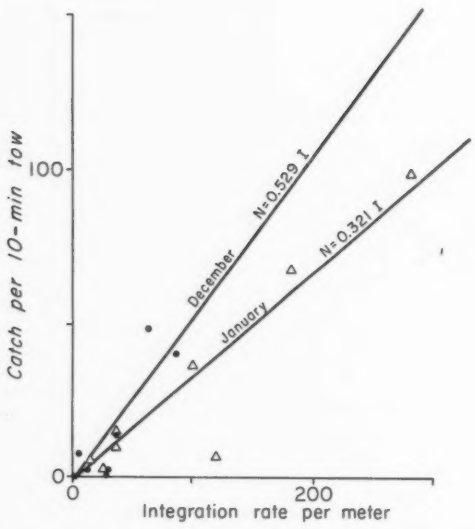


FIG. 4. Relation between numbers of juvenile sockeye salmon and adult smelt per 10-min tow and integration rates/meter taken at night, December 1968 (●) and January 1969 (△).

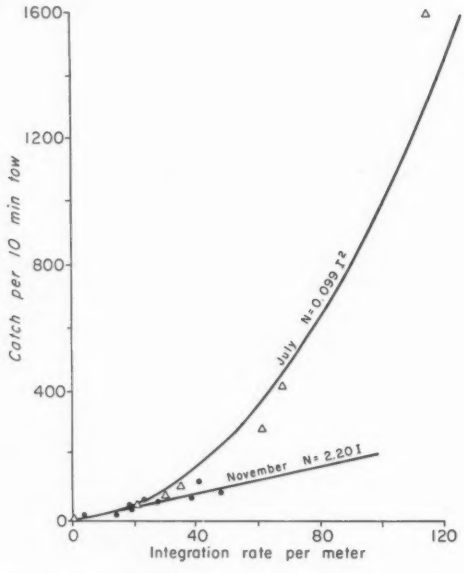


FIG. 5. Relation between numbers of juvenile sockeye salmon and adult smelt per 10-min tow and integration rates/meter taken at night, November 1969 (●) and July 1970 (△).

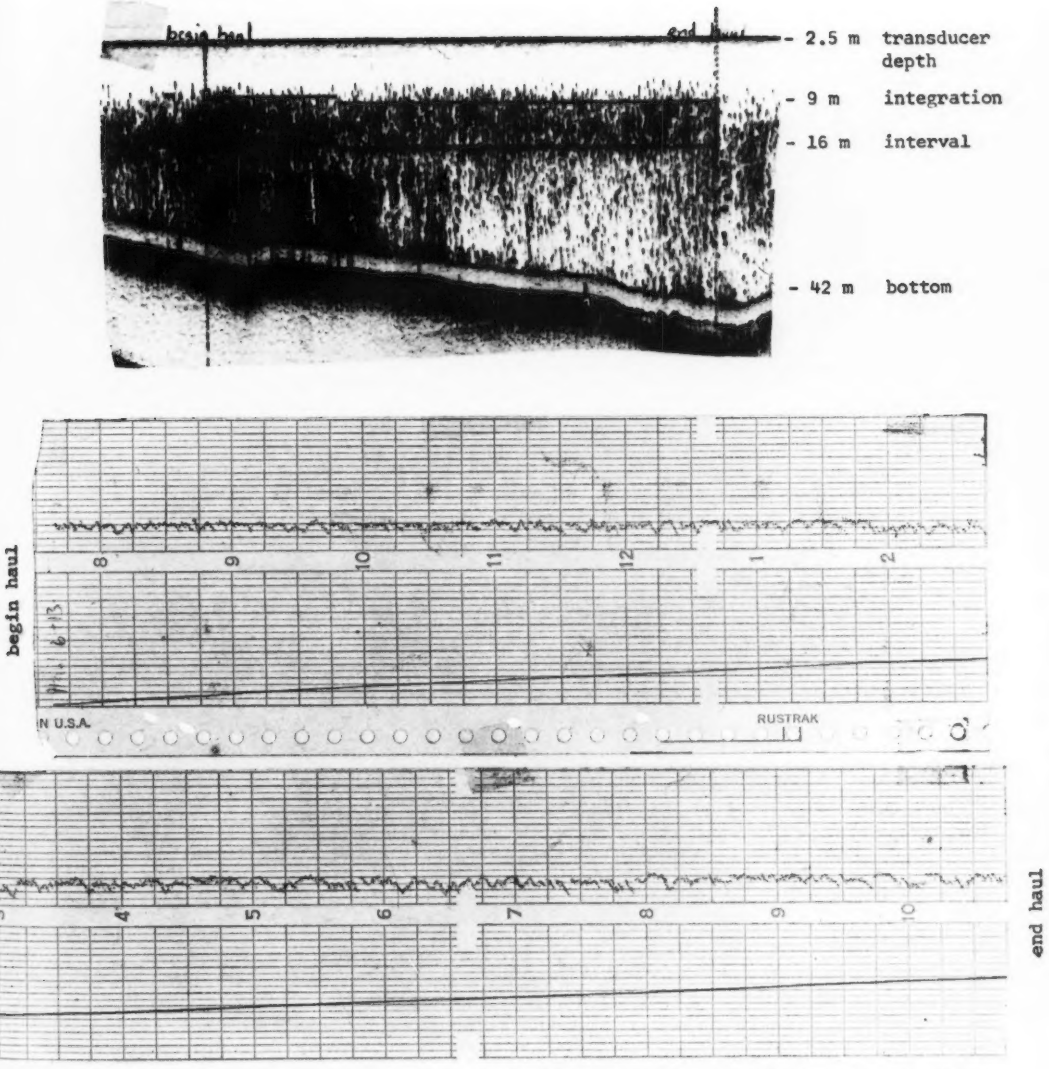


FIG. 6. Echogram and peak detector and integrator output for herring taken at night in Port Orchard, November 1968.

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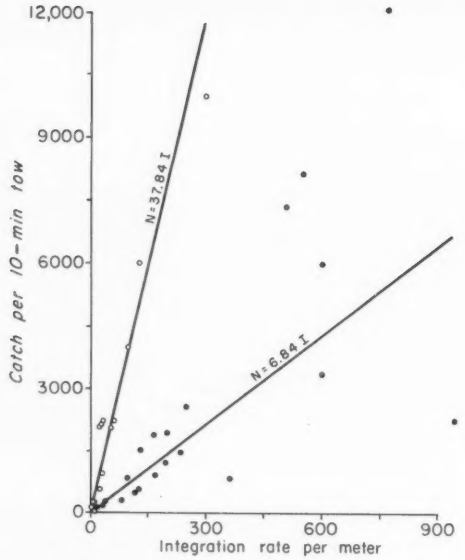

FIG. 7. Relation between catches of herring per 10-min tow and integration rates/meter, day and night, September 1968 to January 1969. Data taken during the day designated by open circles.

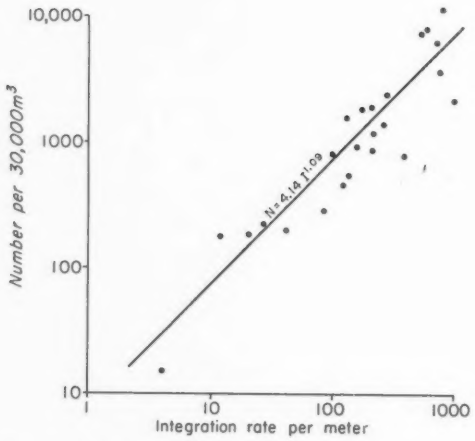

FIG. 8. Relation between catches of herring per 10-min tow and integration rates/meter taken at night, September 1968 to January 1969.

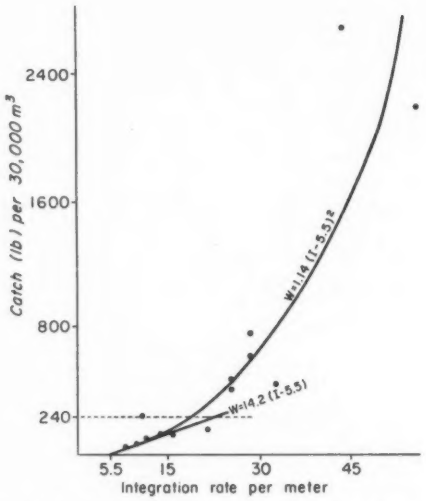

FIG. 9. Relation between catches of fish per 10-min tow and integration rates/meter for hauls containing primarily adult hake taken from Port Susan at night, February to April 1969.

grator output were proportional to intensity, a 20 log R TVG would be the appropriate depth correction to relate density and integration for both multiple and individual targets (Thorne MS 1970).

The appropriate acoustic equipment for population studies may vary depending on the circumstances. When fish are dispersed so that almost all targets can be resolved as individuals, then reasonably simple and inexpensive equipment such as a voltage integrator can be used to obtain a satisfactory population estimate (Thorne and Woodey 1970). When high densities or deep depths result in a prevalence of multiple targets, more expensive and sophisticated equipment, such as $(\text{voltage})^2$ integrator or computer processing systems is probably necessary for accurate results to be obtained.

Acknowledgments

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Estimation of the Pacific Hake (*Merluccius productus*) Population in Port Susan, Washington, Using an Echo Integrator[1]

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THORNE, R. E., J. E. REEVES, AND A. E. MILLIKAN. 1971. Estimation of the Pacific hake (*Merluccius productus*) population in Port Susan, Washington, using an echo integrator. J. Fish. Res. Bd. Canada 28: 1275–1284.

Echo integrators developed at the University of Washington were utilized for estimation of the size of a commercially exploited spawning stock of Pacific hake (*Merluccius productus*) in Port Susan, Washington, in 1969 and 1970. A simple voltage integrator was used in 1969, and a voltage-squared integrator was used in 1970. This change increased the objectivity of the technique, and enabled estimation of confidence intervals. Both population estimates were based on the results of echo integration over a number of transects distributed over the survey area. The integrators were calibrated against net hauls on the assumption of 100% net efficiency. An apparent decrease in the spawning stock size between 1969 and 1970 was indicated by the acoustical census and was supported by a decline in the commercial fishing catch rates between years. Annual catch and age data indicate that this difference apparently is not due to overfishing. The difference probably resulted from variation in the proportion of the total stock that was present in Port Susan at the time of maximum concentration. The major source of variability in the estimates for both years was associated with the net haul calibration. Alternate methods of calibration and improvements in the technique are discussed.

THORNE, R. E., J. E. REEVES, AND A. E. MILLIKAN. 1971. Estimation of the Pacific hake (*Merluccius productus*) population in Port Susan, Washington, using an echo integrator. J. Fish. Res. Bd. Canada 28: 1275–1284.

Dans le but d'estimer l'abondance des merlus pêchés commercialement au large de Port Susan, Washington, en 1969 et 1970, l'auteur a utilisé les intégrateurs d'échos mis au point à l'université de Washington. Il a employé en 1969 un simple intégrateur de voltage et, en 1970, un intégrateur de voltage au carré. En plus d'améliorer l'objectivité de la technique, le changement a permis d'établir des limites de confiance. L'auteur a estimé les deux stocks à partir des résultats de l'intégration d'échos obtenus suivant un quadrillage systématique de l'aire d'observation. Pour l'étallonnage des intégrateurs contre la prise du filet, il a supposé une efficacité du filet de 100%. Le recensement acoustique a révélé un déclin apparent des reproducteurs de 1969 à 1970. La diminution des prises commerciales confirme ce fait. Les statistiques de captures annuelles et les relevés d'âges nous portent à croire que ce déclin n'est pas dû à une surpêche. Il est probablement attribuable aux variations de la proportion du stock entier qui se trouvait dans le détroit de Puget à l'époque de concentration maximale. La source principale de variabilité des estimations des deux années est associée à l'étalonnage des captures du filet. L'auteur considère des méthodes de calibrage alternatives et diverses amélioration à la technique.

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ECHO surveys have been widely used for estimation of the relative abundance of fish in areas of the sea (Cushing 1967). Most echo surveys have been based either on counts of individual echogram marks or on the size and darkness of echo traces from schools. These procedures are often subjective

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because of the difficulties in interpretation of echograms and provide only a relative measure of abundance. Several electronic apparatus for quantifying returning echo signals have been developed, including a pulse counter (Mitson and Wood 1961), a pulse length counter (Carpenter 1967), and an echo integrator (Dragesund and Olsen 1965). Despite these advances, signal-processing equipment has been seldom used in estimations of fish stocks (Truskanov and Scherbino 1966; Cushing 1968). One of the obstacles to absolute estimation of fish stocks acoustically is the natural complexity of the marine environment. The distribution of most fish stocks occurs over a large area with intermingling among stocks, so that identification and separation are difficult.

The distribution of Pacific hake (*Merluccius productus*) in Puget Sound makes this stock ideally suited for acoustical censusing. Although the hake are present throughout greater Puget Sound, large aggregations apparently occur only in Saratoga Passage and Port Susan (Fig. 1). Evidence from the commercial fishery suggests that hake from many areas of Puget Sound migrate to Port Susan each winter to spawn and then disperse after spawning; sexually maturing hake comprise over 90% of the pelagic fish biomass in Port Susan from early February to May, but are not found there in commercial quantities during the remainder of the year.

Puget Sound hake are apparently genetically distinct from the oceanic stock that inhabits Washington's coastal waters each summer. Utter (MS 1969) found significant differences in enzyme systems, and Nelson and Larkins (1970) reported that growth rates of Puget Sound fish were much slower than coastal hake. Utter (MS 1969) found strong evidence of genetic isolation.

An industrial-use hake fishery was initiated in Puget Sound in November 1965. It operates mainly in Saratoga Passage from October to December and in Port Susan from January to May or June. Annual landings have averaged over 8.5 million lb through the 1969–70 season and have ranged from 6.3 to 10.6 million lb. Over 80% of the annual total has come from Port Susan (Millikan 1970).

An acoustical census of the hake in Port Susan was undertaken in 1969 and 1970 with echo integrators developed at the University of Washington (Lahore MS 1969; Thorne MS 1970). The objectives of the study were to obtain an estimate of the spawning population in Port Susan for management purposes, to evaluate the potential and efficiency of the echo integrator as a tool for estimation of fish population size, and to develop sampling techniques for future acoustical enumeration of hake in Port Susan.

Materials and Methods

The Echo Integrators

An echo integrator measures the total voltage of the fish echoes received from a given depth interval and sums these voltages over time. Two different echo integrator systems were used during the 2 years of survey. In 1969, a Simrad EH2E sounder with a frequency of 38 kHz and a pulse length of 0.5 msec was used with the voltage integrator described by Lahore (MS 1969) and Lahore and Lytle (1970). A time-varied-gain (TVG) of 20 log R in decibels (db) was included in the electronics of the integrator. In 1970, a Simrad EK38A Scientific Sounder with a frequency of 38 kHz and a pulse length of 0.6 msec was used. A 20-log R TVG was an integral part of the sounder. The integrator contained a squaring circuit designed to correct for multiple targets that occur at higher densities. Thus, the integrator summed the squares of the voltages from the fish echoes, and integrator output was proportional to integrated acoustic intensity (Thorne MS 1970; Thorne 1971). The depth gate and integrating circuits remained unchanged (Lahore MS 1969; Lahore and Lytle 1970).

Calibration Procedures

The population estimations in both 1969 and 1970 were based on calibration of the integrators with net hauls that were assumed to have a catching efficiency of 100%. Attempts were made in 1970 to measure the target strength of hake directly, thus eliminating the need for calibration with net hauls, but were unsuccessful because of failure of auxiliary equipment. The procedure for determination of the relation between integrated echo voltage and density of fish, and the results of calibration on hake in 1969 are given in Thorne (1971). Procedures were similar in 1970 except for the use of a different midwater trawl. The net used in 1969 is described in Thorne (1971). The net used in 1970 was constructed of 3-inch mesh monofilament nylon webbing in the wings and body and 1⅝-inch mesh multifilament nylon webbings in the codend. Horizontal and vertical wingtip openings were about 15 and 3 m, respectively. The net filtered approximately 45,000 m^3 in a 10-min haul, compared to 30,000 m^3 for the net used in 1969.

Survey Area and Methods

The physiography of Port Susan is ideally suited to acoustical census. It is a long narrow basin with steep sides, smooth bottom, and a relatively uniform depth (between 100 and 120 m).

The survey area was chosen to include the entire known spawning area. The 10-fath (20-m) contour of the bay was selected as the periphery since in preliminary surveys spawning hake were not found in shallower

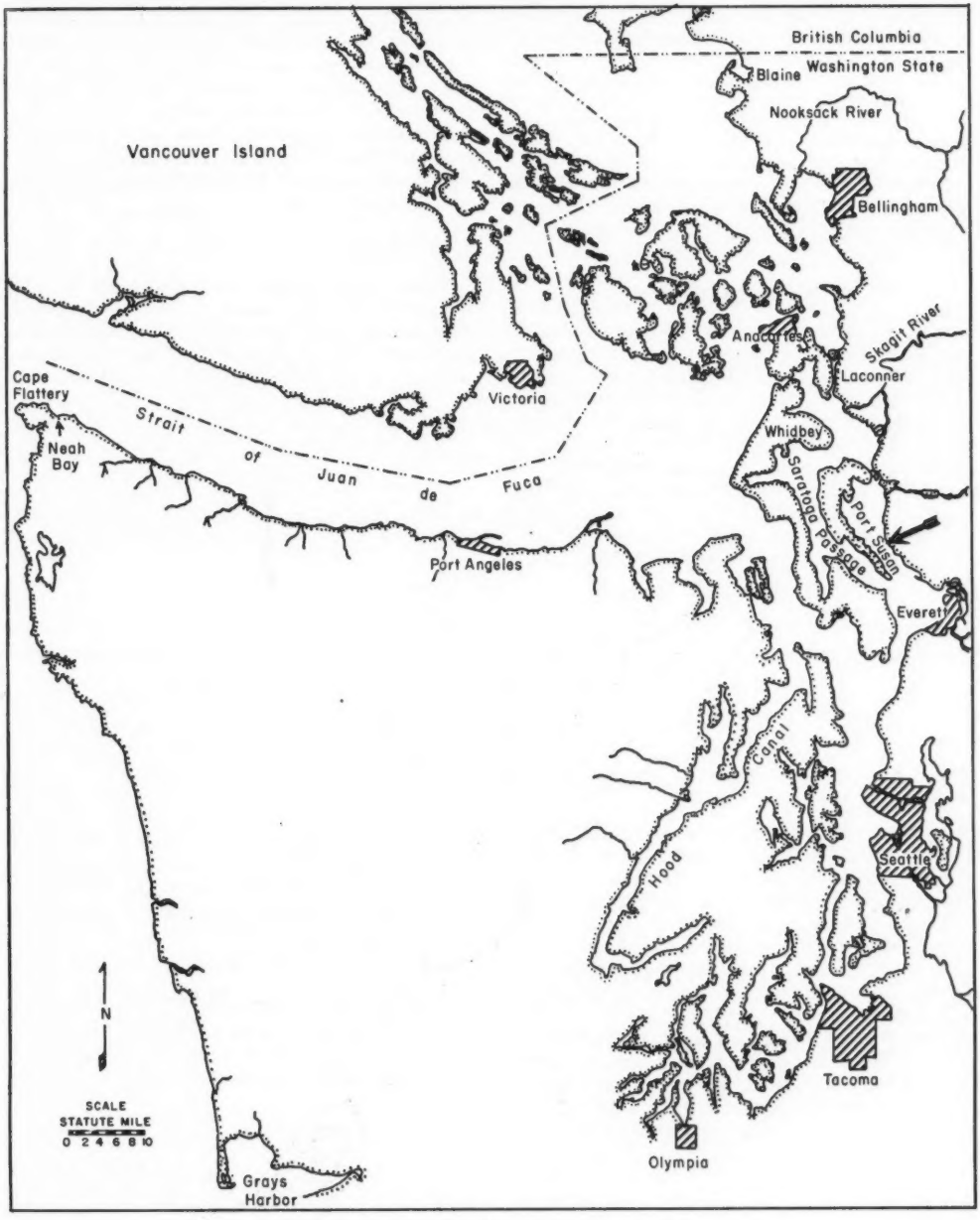

FIG. 1. Puget Sound, showing survey area.

water. The survey area was divided into nine subareas. Two survey tracklines, one in a northerly direction and one in a southerly, were plotted. Each trackline consisted of nine oblique transects, one within each subarea (Fig. 2).

The prescribed trackline was cruised with the integrator aboard the University of Washington's research vessel *Commando*. The integrated interval of the water column was from 35 m below the surface to 1–3 m above bottom. Boat speed was a constant 10 knots.

Integrator and peak detector output were recorded on voltage meters and on a 2-channel Rustrak chart recorder. At the end of each transect, the total integrated voltage and the duration of the transect were noted.

Biomass Estimates

The population of hake in Port Susan was first estimated in winter and spring 1969. The main goal of this survey was to determine the feasibility of acoustic assessment. The second annual survey, in winter and spring 1970, was made with the improved integrator with the squaring circuit. Major emphasis was put on evaluating the precision of the biomass estimates obtained. The basic method used in both surveys to estimate biomass was that of simple proportions. Densities of fish (lb/m³) estimated from integration rates were raised by the volume of water (m³) in the survey area to obtain total pounds of fish.

1969 Estimates

Three survey cruises were conducted in 1969 aboard the M/V *Commando*, one each in February, March, and April. The results of calibration from net hauls taken at night during the three cruises are given in Fig. 3 (from Thorne 1971). On the basis

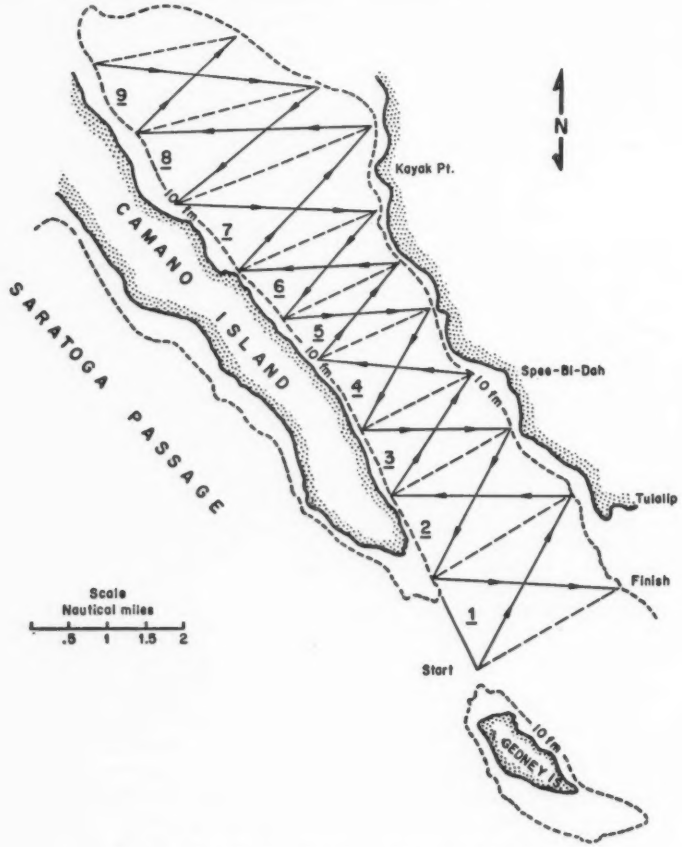

FIG. 2. Port Susan, showing subareas and tracklines.

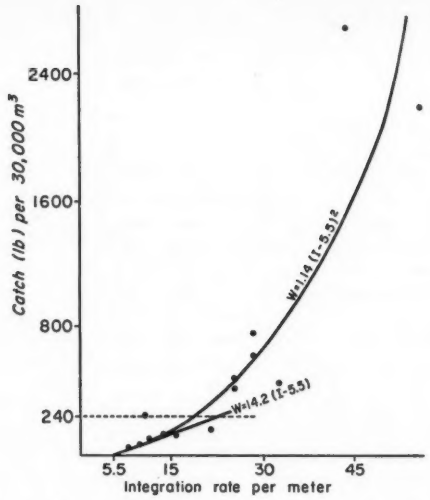

FIG. 3. Relation between density of hake and integration rate per meter from net hauls in Port Susan in 1969.

of the data and theoretical considerations, integration-rate values were divided into two categories: those with integration rates/meter below 25, which were considered to represent individual targets; and those with rates of 25 or above, which were considered to represent multiple targets (Thorne 1971). Two calibration relations were necessary because high-density schools exhibited a patchy distribution and caused heterogeneity in density within the integrated depth intervals of the survey transects. The relation between fish density and integration rate/meter for the lower integration values was determined by least squares to be

$$w = 14.2\ (\mathrm{I}-5.5),$$

where w is units of pounds per 30,000 m³; I is the integration rate/meter; and 5.5 is the noise level. The relation for densities in the multiple-target region was determined, also by least squares, to be

$$w = 1.14\ (\mathrm{I}-5.5)^2.$$

Twelve tracklines were run during each cruise, six in a northerly direction and six in a southerly (Fig. 2). The integration values from the transects contain components from both individual and multiple targets. These two components must be separated for estimation of abundance, since the calibration relation depends on whether or not the targets are individually resolved. Thus, the estimator of biomass used in 1969 necessarily consisted of two terms. For the ith transect:

$$\mathrm{W}_i = \mathrm{W}_{\mathrm{S}i} + \mathrm{W}_{\mathrm{M}i},$$

where $\mathrm{W}_{\mathrm{S}i} = w_{\mathrm{S}i}\,\mathrm{A}_i$ = biomass for single targets (S); $w_{\mathrm{S}i} = \hat{b}_{\mathrm{S}}\,\mathrm{I}_{\mathrm{S}i}$ = lb/30,000 m²; $\mathrm{I}_{\mathrm{S}i}$ = [volts (from single targets)/600 sec/m] D_i obtained over the ith transect; D_i = mean integration depth interval of the ith transect (m); $\hat{b}_{\mathrm{S}}$ = single-target slope coefficient relating volts/600 sec/m to pounds/30,000 m²; A_i = surface area represented by the ith transect.

Note that the depth, D_i, is inherently a part of the transect integration, $\mathrm{I}_{\mathrm{S}i}$, since the integration depth intervals of the survey transects extend over the entire range inhabited by the population. Thus, the transect integration is multiplied by the surface area rather than the volume in order to determine biomass.

The second term is the additional biomass from multiple targets (M):

$$\mathrm{W}_{\mathrm{M}i} = \left(\sum_{j=1}^{n} w_{\mathrm{M}ij} d_{ij} \mathrm{P}_{ij}\right) \mathrm{A}_i,$$

where

$$w_{\mathrm{M}ij} = \hat{b}_{\mathrm{M}} \mathrm{I}_{\mathrm{M}ij}^2 - b_{\mathrm{S}} \mathrm{I}_{\mathrm{M}ij}$$
$$= \mathrm{lb}/30{,}000\ \mathrm{m}^2$$

where $\mathrm{I}_{\mathrm{M}ij}$ = volts (from multiple targets)/600 sec/m estimated for the jth multiple-target school; b_{M} = intercept coefficient for the multiple-target calibration relation; d_{ij} = depth range of the jth school (m); P_{ij} = proportion of the transect duration occupied by the jth school; and n = number of multiple-target schools.

Integration values were corrected for noise level and were time-adjusted to 600 sec before being substituted into these equations. Estimated biomass values for the transects in each trackline were summed. The means of trackline biomass estimates for the three surveys are 24.4, 50.8, and 12.0 million lb for February, March, and April, respectively. These figures represent the total fish biomass in the area and within the integration depth interval. Net haul data indicated that at the time of the surveys 92% of the fish biomass was composed of hake. When this figure is taken into account, the revised estimates are 22.4, 46.7, and 11.0 million lb for the 3 months (Table 1).

TABLE 1. Monthly estimates of biomass of hake in Port Susan, Washington, February through April 1969.

Month	Sample size	Mean (*million lb*)	Range	SD
Feb.	12	22.4	14.4–28.2	1.32
Mar.	12	46.7	29.3–65.1	2.82
Apr.	12	11.0	7.3–18.1	1.06

Realistically, variance estimates of the population must include not only sampling error associated with transecting but also error associated with the estimation of density from integrated voltages. The complicated nature of the estimator used in 1969, and the fact that subsequent improvements in the system rendered this estimator obsolete, made it difficult to justify a serious attempt at variance estimation.

Without question, however, the major component of variance in the population estimate came from the net haul calibration. The 95% confidence intervals on the regression constants for the two calibration equations are both ±50% of the values of the constants. In comparison, the 95% confidence intervals on the mean of the 12 trackline estimates in March are about ±15%.

Another important source of error in the 1969 survey procedure came from the subjectivity involved in estimating the I_{Mij}. The occurrence of schools of fish of high density within the integration interval could be objectively detected with the peak detector. The actual determination of the vertical extent of the multiple target densities and the partitioning of the total integration within the integration depth interval between components from the high-density school and the remainder of the integration interval was largely subjective and based on the darkness of the traces. An example of an echogram and peak detector output is shown in Fig. 4. The occurrence of a school of fish with an integration rate per meter greater than 25 for a duration of 90 sec is indicated in the example by the peak detector and outlined on the echogram. The total integration occurring during the 90 sec was 350 v. The depth interval of the outlined school was estimated at 17 m in an integration interval of 57 m. On the basis of the darkness of the trace the integration rate outside the school was given a value of 20 v/600 sec per m. This results in an integration from outside the school of 120 v[(20 v/600 sec per m)(90 sec)(40 m)], leaving 230 v as the portion from the school, or a rate per meter (I_M) of 90 v/600 sec per m, or a density of 1 lb/m³ (1 fish/2 m³). This method of estimating integration rates for high-density schools was unavoidable with the system used in 1969. However, this problem was overcome because of improvements in the integrator system prior to the 1970 survey.

1970 ESTIMATE

Surveys were conducted as in 1969 but not monthly; four cruises were made during the months of March and April in an attempt to delineate the suspected peak of hake abundance within the bay more precisely. We were not successful in conclusively establishing the time of peak abundance, since the first survey produced the highest estimate. However, catch rates from the commercial fishery indicate that no major peaks in abundance occurred previous to the first cruise.

Because of the incorporation of a squaring circuit into the integrator system, the relation between fish density and integration rate per meter could be considered linear. A linear calibration model without a constant term was assumed:

$$w = \hat{b}\mathrm{I},$$

where I = (volts)²/sec per m; w = lb/m³; and $\hat{b}$ = estimated slope coefficient.

The model was fitted to data that had been collected in the same manner as the calibration data taken in 1969. System noise was subtracted from the integration rate data prior to fitting the calibration model to it. Data points, representing simultaneous net hauls and integration runs, and the fitted line are shown in Fig. 5. The slope coefficient is estimated as 8.92 with an estimated variance of 6.791 (6 degrees of freedom).

The large variability in net catch is partially the result of trawling during the daytime. Thorne (MS 1970) showed that the relation between integration and net catch of hake was similar for day and night, but that the catch variability was far greater during the day. The linear relation between density and integration is not apparent from the net catch data because of the high variability and small range of density, but the linear nature of the relation has been well established from theoretical considerations and independent measurements (Thorne MS 1970; Moose and Eherenberg 1971).

The linear calibration function made possible a simplified estimator of population biomass and a simplified procedure for estimating variances. Additionally, with the 1970 estimator, methods of stratified sampling can readily be employed for more efficient estimation. If we assume that fish densities are randomly sampled, for any one time period, then

FIG. 4 stratin

Thorne

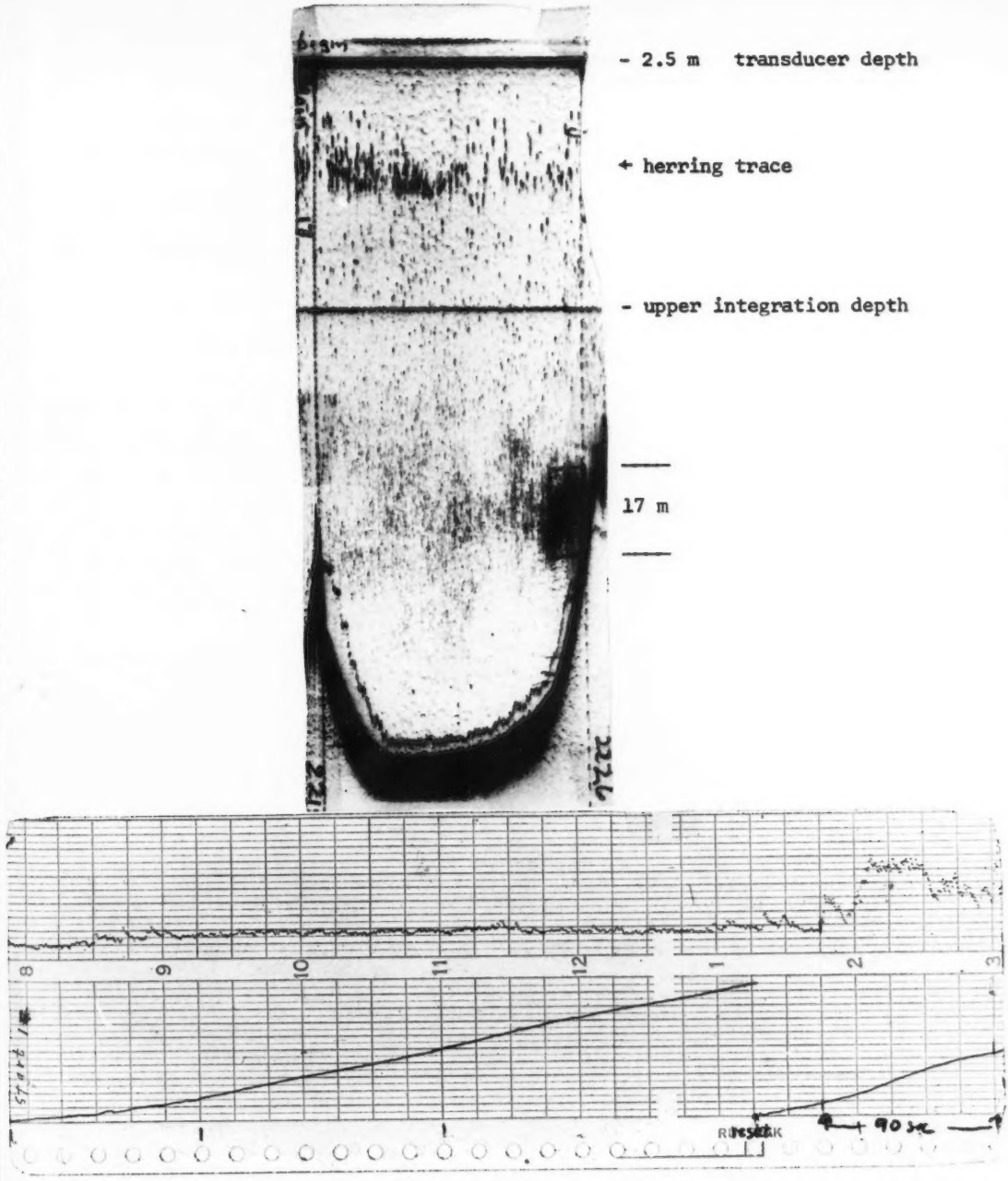

FIG. 4. Echogram and peak detector and integrator output from transect 16, trackline 4, in Port Susan, February 1969, demonstrating occurrence of high densities of hake.

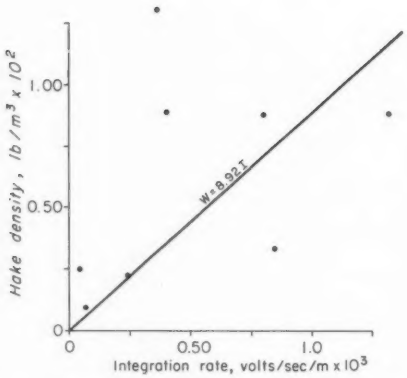

FIG. 5. Relation between catch of hake per 10-min tow and integration rate per meter in Port Susan in 1970.

$$W = \sum_{h=1}^{L} A_h \bar{w}_h,$$

where A_h = area of hth density stratum (m^2); L = number of strata; and

$$\bar{w}_h = \hat{b}\bar{I}_h = \text{lb/m}^2,$$

where $\hat{b}$ = linear slope coefficient; and

$$\bar{I}_h = \frac{\sum_{i=1}^{n_h} I_{hi}}{n_h},$$

where I_{hi} = (v/sec per m)$_i$ × D_{hi} = integration rate/m × depth (m) over the ith transect in hth stratum; D_{hi} = mean depth interval of ith transect in hth stratum; n_h = number of transects in hth stratum.

The I_{hi} values were corrected for noise level and for nonhake biomass before calculations were made. The latter quantity was estimated as 7% from the 1970 net haul data.

Variances were computed using

$$v(W) = \sum_{h=1}^{L} A_h^2 v(\bar{w}_h)$$

where

$$v(\bar{w}_h) = \hat{b}^2 v(\bar{I}_h) + \bar{I}_h^2 v(\hat{b}),$$

where

$$v(\bar{I}_h) = \frac{s_h^2}{n_h},$$

where

$$s_h^2 = \frac{\sum I_{hi} - \frac{(\sum I_{hi})^2}{n_h}}{n_h - 1}, \text{ with } n_h - 1 \text{ degree of freedom.}$$

The estimator for $v(\bar{w}_h)$ was derived by the delta method (Deming 1946), on the assumption that errors in $\bar{I}_h$ and $\hat{b}$ are independent. The assumption of independence was also necessary in estimating the variance of the population total, $v(W)$, since estimates of the covariance between the $v(\bar{w}_h)$ were not available. It is likely that these errors are correlated, since a common value for $\hat{b}$ was used in their estimation. Thus, estimates of the $v(W)$ must be considered as minimum values. Sampling fractions were approximately 10% or less for all strata and cruises; thus, the finite population correction was ignored in the calculation of $v(W)$.

A normal distribution was assumed, and the 95% confidence intervals were calculated by the formula

$$\text{C.I.} = W \pm t_{95,df} \sqrt{v(W)}$$

where $t_{95,df}$ = "t" value associated with df (= $\sum_{i=1}^{L} (n_h - 1)$ + L[df for $v(\hat{b})$]) degrees of freedom.

The estimates of hake biomass and their confidence limits are shown in Table 2.

Discussion

Sampling Considerations

The use of a stratified estimator has two advantages over the method used in 1969. Sampling error is usually reduced, and a framework for evaluating sampling strategy becomes available. When stratified sampling is appropriate, the most efficient estimate is achieved through optimum allocation of sampling effort (Cochran 1963), whereby both desired variances and cost of obtaining them are taken into account. This method was attempted for Port Susan hake. On the first cruise, after two tracklines had been completed, stratum boundaries based on density were set up according to Cochran's rules (1963). In general, optimum allocation means taking more samples in strata that are larger, are more variable with respect to the quantity being measured, and in which sampling is cheaper. In our instance, the cost per transect did not vary between strata. Thus, only information on stratum sizes and variances was required for optimum allocation (see Cochran equation 5.20). Each

Table 2. Estimates of hake biomass in Port Susan, Washington, in 1970.

Cruise	Date	Hake biomass (*million lb*)	95% confidence limits	
			Upper	Lower
1	Mar. 7, 8	13.5	19.7	7.4
2	15, 16	10.8	15.5	6.2
3	25	4.3	6.5	2.0
4	Apr. 1	5.7	8.8	2.6

subarea was assigned to a stratum based on the integration rates obtained from transects in the subarea. Variances of integration rates were then estimated from the transect data, and stratum sizes were obtained by summing the areas (in m^2) of the appropriate subareas. Then, for a fixed total number of transects, the number to be run in each stratum for the remainder of the cruise was determined. However, it soon became apparent that the distribution of densities as indicated by integration rates was not stable as sampling continued. This instability did not appreciably alter the basic heterogeneity of the population, but did make it difficult to predict stratum sizes from the first tracklines of any cruise. Thus, any attempt at optimum allocation of sampling effort was invalidated. Moreover, errors in strata sizes can introduce serious bias into the estimate of total population (Cochran 1963). The approach taken was to delay stratification until the cruise was completed. Subareas were delegated to strata based on the average value of integration rates over all the transects taken in the subarea, which provided a more precise delineation of strata. According to Cochran, the method of stratification after sampling approaches the precision of proportional allocation (usually less than that of optimum allocation) as the number of samples in each stratum increases and when errors in stratum size can be ignored.

For practical purposes, errors in the determination of stratum size can be considered insignificant. Mean densities of some subareas had confidence intervals (95%) that somewhat overlapped the stratum boundary. Over the four cruises, this overlap, expressed in terms of the width of the confidence interval, averages about 19%. This percentage indicates that there is some chance of misidentifying the stratum to which any particular subarea belongs, but it was not very great for these surveys. In some instances the width of the confidence interval probably reflects small samples. For example, in the last two cruises with smaller numbers of transects, the distribution of densities exhibited as high or higher a degree of stability as those of the first two cruises, but the average amount of overlap of confidence intervals was higher. Further, reassigning questionable subareas to strata had little effect on recomputed population estimates. For the four cruises, differences in population estimates averaged 5%, whereas the precision of these estimates averaged 49%. These problems are outweighed by the benefits of stratified sampling. The average gain in precision due to reduction of sampling errors through stratified sampling (Cochran 1963 p. 137), as compared to simple random sampling, was estimated at 29%.

As suggested by Fig. 5, the conversion of integration rates to fish density is the major source of variation in the population estimate. Error due to the "beta" component of the variance equation ranged between 2 and 23 times the magnitude of the "sampling error" component and averaged 11 times greater in magnitude for the four cruises. The large "beta" component results partially from the necessity to base this estimate on a small number of net hauls. Original plans called for basing the calibration on direct measurement of target strength and using net hauls only for determining species composition. When we realized that this objective could not be met, it was too late in the season to obtain additional net-haul data.

Concerning sampling intensity for estimating density of hake in Port Susan at any one time, a series of about 40 transects (20 per stratum) is indicated for the range of densities encountered in 1970. Cochran suggests that sample sizes should be 20 per stratum for achievement of a level of precision equal to that of proportional allocation when stratification after selection of the sample is employed. From past experience, this level of sampling appears adequate. Reexamination of transect data from cruises 1 and 2 (neglecting the error due to calibration) shows that the first 36 transects from cruise 1 gave a precision of $\pm 15\%$. On cruise 2, the first 36 transects produced a precision of $\pm 16\%$ and an additional 39 transects only reduced it to $\pm 11\%$. Sampling effort on cruises 3 and 4 was about half that for the first two cruises, yet overall levels of precision for all cruises were similar (Table 2). However, if future ranges of densities encountered increase and more strata are used, additional transects will be needed. The predicted 40 transects per cruise will also need to be reevaluated as the error of calibration is reduced through additional field work.

Sources of Bias in the Estimates

Although the estimators used on the two annual surveys differ and have their own special characteristics, there are certain problems associated with the survey method that affect both estimates in the same ways. Two possible sources of bias are: (1) additional fish outside the boundaries of the area covered, and (2) errors in assumptions associated with net-haul calibrations.

The areal coverage of Port Susan was very good. Because of the simple geometric shape of the area and the steep-sided feature of the basin, the survey transects covered the area virtually from shore to shore and from one end to the other. The main source of error in the survey coverage of Port

Susan is associated with fish on the bottom. The distribution of fish extended to the bottom, and in many instances the greatest densities were just above the bottom. The lower integration interval was between 1 and 3 m above the bottom, so fish in the water near the bottom as well as fish on the bottom were not included.

The calibration of the integrator was based on net hauls in both years and we assumed that the nets used were 100% efficient. Any tendency for fish to avoid the net, or for the gear to herd fish into the catch zone, will bias the estimate.

Management Implications

The results of the population estimates indicate the general size of the spawning stock of hake in Port Susan as well as a difference between years. Although variability in the estimates and the sources of bias are considerable, the results at this stage do lead to some conclusions relating to management of the stock, especially when evaluated in concert with existing biological and catch data. For example, the apparent decrease in spawning stock size between 1969 and 1970 that was indicated by the acoustical census was also indicated by a 33% decline in catch rates between years. Although the difference between years cannot be quantified with high confidence, the inference of a smaller stock size is probably valid.

Overfishing does not appear to be occurring, despite the observed differences in stock size. It is possible that the small estimate in 1970 resulted partly from hake remaining outside the survey area rather than entirely from a decrease in population size. Spawning stock estimates obtained in Port Susan do not account for fish outside the census area. Exploratory data suggest that it is unlikely that other major spawning areas exist, but the percentage of Port Susan spawners outside the survey at the time of survey is unknown. Biological and catch data indicate that males enter the area early and remain throughout the season, whereas females remain in the area for a relatively short duration and emigrate soon after spawning. A predominance of males (average 65%) suggests that females not fully mature remained outside the area.

These data suggest that fishing may be occurring on a larger population than was estimated for Port Susan from the survey data. In addition, catch levels and calculated mortality rates in 1970 were similar to past seasons and no severe shift in age distribution was noted.

Future Studies

Other major benefits of the 2-year study have been a gain in experience with acoustical censusing and knowledge as to areas for improvement in the technique. Three specific areas of improvement and research are suggested: (1) refinement of the relation between integrated voltage and fish density, (2) standardization and absolute calibration of acoustic gear, (3) examination of the basic assumptions concerning the spawning behavior of the Puget Sound hake stock.

The average echo strength of a fish depends on its target strength and the characteristics of the acoustic equipment. If these two factors are known, the average echo strength can be determined and the relation between fish density and integrated voltage derived, if one assumes that sound absorption, shading, and multiple scattering effects at high densities are negligible. This assumption is probably correct for the range of densities of hake in Port Susan. During this study the average echo strength of a fish was determined indirectly by comparison of net catch with integrated voltage. This procedure is not entirely satisfactory because of the large variability associated with net catch and the assumptions concerning net efficiency.

Methods of direct target strength measurement have been described, such as that of Midttun and Nakken (1968). These methods require resolution of individual targets. Direct target strength measurement was attempted on the hake in 1970 but was unsuccessful because of difficulties in finding low densities where individual targets could be examined, combined with failure of magnetic taping equipment. Despite the failure of the first attempt, the methods appear promising and will be applied in future studies so that estimates will be in absolute numbers.

Since this study involved the development of a new technique, standardization was impossible. The basic instrument, the integrator, underwent major modification between the 2 years. The resulting degree of uncertainty in the comparison of the estimates for the 2 years underscores the importance of standardization and absolute calibration of acoustic equipment as early as possible in yearly studies. With proper electronic and acoustic calibration procedures, the echo abundance of hake can be directly compared from year to year, even though the target strength, and therefore the absolute abundance, may be uncertain. Further, the estimates can be refined when more information on target strength of the hake becomes available as a result of subsequent research.

Catch statistics and biological data indicate that a major reason for the smaller estimate in 1970

was due to a smaller portion of the stock spawning in Port Susan. Variability from this source is critical in the estimate, since the primary assumption upon which the stock size estimate is based is that most of the Puget Sound stock of hake is present in Port Susan at some time during the winter. Variability in the timing of the spawning and in the maximum proportion of the stock present in Port Susan from year to year should be studied so that the limitations associated with this assumption can be determined.

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Methylmercury in Freshwater and Marine Fishes in New Brunswick, in the Bay of Fundy, and on the Nova Scotia Banks

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American eel (*Anguilla rostrata*), chain pickerel (*Esox niger*), white perch (*Morone americana*), yellow perch (*Perca flavescens*), brook trout (*Salvelinus fontinalis*), and Atlantic salmon (*Salmo salar*) contained 0.07–2.08, 0.27–1.58, 0.75–1.07, 0.20–1.05, 0.08–0.13, and 0.09 ppm of methylmercury, expressed as mercury on wet weight basis, respectively. With only two exceptions, levels of methylmercury in marine fish were below 0.13 ppm. Some of the freshwater sampling locations could be directly associated with an industrial activity in the area, whereas others suggested pollution by airborne mercury. However, the concentration of methylmercury in eels from a lake in New Brunswick did not change over a period of 46 years, indicating that elevated levels of mercury may in some instances reflect the natural situation.

ZITKO, V., B. J. FINLAYSON, D. J. WILDISH, J. M. ANDERSON, AND A. C. KOHLER. 1971. Methylmercury in freshwater and marine fishes in New Brunswick, in the Bay of Fundy, and on the Nova Scotia Banks. J. Fish. Res. Bd. Canada 28: 1285–1291.

L'anguille d'Amérique (*Anguilla rostrata*), le brochet maillé (*Esox niger*), la perche blanche (*Morone americana*), la perchaude (*Perca flavescens*), l'omble de fontaine (*Salvelinus fontinalis*) et le saumon atlantique (*Salmo salar*) contiennent respectivement 0.07–2.08, 0.27–1.58, 0.75–1.07, 0.20–1.05, 0.08–0.13 et 0.09 ppm de mercure méthylique exprimé en quantités de mercure par unité de poids humide. A deux exceptions près, les échantillons de poissons marins ont des concentrations de mercure méthylique inférieures à 0.13 ppm. Quelques stations d'échantillonnage en eau douce peuvent être associées directement aux opérations industrielles du voisinage, alors que d'autres suggèrent une pollution aérienne. Par ailleurs, la concentration de mercure méthylique des anguilles d'un lac du Nouveau-Brunswick n'a pas changé depuis 46 ans, ce qui indique que de hautes concentrations de mercure peuvent, dans certains cas, être le résultat de conditions naturelles.

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THIS paper reports on the methylmercury content of some freshwater fishes in New Brunswick and of marine fishes from the Bay of Fundy and the Nova Scotia Banks. Methylmercury was also determined in two museum specimens, caught in New Brunswick in 1924. Inorganic mercury was determined in several samples of surface water and of precipitation.

Mercury pollution was first detected in Japan (Irukayama 1966), then in Sweden (Johnels et al. 1967), and recently in Canada (Wobeser et al. 1970; Bligh MS 1970), and in the United States (Anon. 1970a, b, c), but no data on the concentration of methylmercury in fish from Canada and the United States were reported. It has been shown in Sweden that most mercury in fish (on the average 92% in freshwater and 82% in marine fish) exists as methylmercury (Norén and Westöö 1967; Johansson et al. 1970). Analytical chemistry of methylmercury was recently reviewed (Fishbein 1970).

Mercury enters the environment largely as waterborne mercury, but data from Sweden indicate that airborne mercury can be a substantial source of pollution (Tejning MS 1967). Waterborne mercury passes from solution or suspension into sediments. Organisms transform mercury into methylmercury

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compounds and dimethylmercury. In this process mercury passes from sediments into water and as dimethylmercury enters the atmosphere (Jernelöv 1969). The presence of mercury in the air was demonstrated in several industrialized areas (Brar et al. 1970; Klein and Goldberg 1970). One of the sources of airborne mercury is coal (Klein and Goldberg 1970). Thus, regardless of the form of discharge, mercury is able to circulate widely in the environment.

Materials and Methods

Freshwater fish were taken from 16 stations from June to September 1970 (Fig. 1). Marine fish were taken from 30 stations in July 1970 (Fig. 2). All samples were frozen immediately and stored frozen until analysis.

Samples of muscle (10 g) from individual fish, or pooled samples from up to 8 specimens, were analyzed according to Westöö, procedure A (Westöö 1968). Museum specimens had been preserved in ethanol. Muscle from these specimens was dried in vacuum (27 psi) at 60 C and 1-g samples were used for analysis. Swordfish protein concentrate was obtained from Dr L. W. Regier, Fisheries Research Board of Canada, Halifax Laboratory, Halifax, N.S.

A Varian 600D gas chromatograph with an electron-capture (tritium, 250 mc) detector was used. A glass column (5 ft × ⅛ inch), containing 10% Carbowax 6000 on Chromosorb W, 50/60 mesh, was operated at

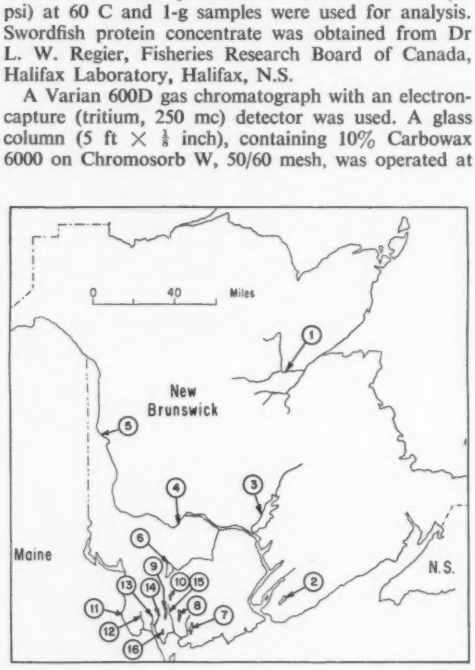

FIG. 1. Freshwater sampling locations: 1 Miramichi, 2 Loch Lomond, 3 Grand Lake, 4 Mactaquac Impoundment, 5 Beechwood Impoundment, 6 Lake Oromocto, 7 Lake Utopia, 8 Digdeguash Lake, 9 St. Patrick's Lake, 10 Clarence Lake, 11 Grand Falls, 12 Dennis Stream, 13 Gallop Stream, 14 Waweig River, 15 Digdeguash River, 16 Chamcook Lake.

FIG. 2. Marine sampling locations.

150 C and a nitrogen flow rate of 70 ml/min. The injector temperature was about 175 C. Under these conditions the retention time of methylmercury was approximately 3 min. The peak area was determined by triangulation. A standard solution of methylmercuric chloride (Alfa Inorganics, Inc.), 1 ng/μliter, in benzene, was used for calibration. The calibration was carried out at least once a week and after every change of the septum. Typical responses of the detector to methylmercury on several consecutive days are presented in Table 1. The recovery of methylmercuric chloride (3.56 and 7.13 μg) in the procedure was 82%.

Confirmations of methylmercury based on the reaction with silver sulfate (Widmark and Jensen (Holden 1970)), by thin-layer chromatography according to Westöö (Westöö 1966), and by high resolution mass spectrometry (Jamieson et al. 1971) were carried out on several samples.

Fish from a given locality were anlyzed as one or several samples. In the latter instance, the range is also reported. Duplicate analyses were run on samples with high methylmercury content. The precision of the method, as indicated by duplicate sample analyses, was ±10%.

TABLE 1. Electron-capture detector response to methylmercury chloride.

Day	Peak area × 2, cm²/ng
1	12.1
3	14.5
4	16.1
8	15.1
9	15.1
11	15.9
12	13.9
15	16.0
16	15.2
17	14.8

Known amounts of methylmercuric chloride (0.07, 0.14, and 0.8–1.1 ppm) were added to several samples. The recovery of added methylmercuric chloride was 79, 100, and 92%, respectively.

Samples of lake waters were acidified immediately after collection with sulfuric acid to pH = 1 (Chau and Saitoh 1970). Rain water and snow were collected in the vicinity of St. Andrews, N.B., over the period October 1970 to February 1971. The rain was collected using plastic funnels (10 inch diam), tightly inserted into 3-liter plastic bottles. The plastic ware was washed before assembling with diluted (1:1) nitric acid and rinsed with distilled and deionized water. Freshly fallen snow was collected in wide-necked plastic bottles using a small plastic bottle as a plunger. The bottles were cleaned inside and outside as described above. Concentration of mercury in water was determined with dithizone (Sandell 1965). Sulfuric acid was added to the sample (500 ml) to a final concentration of 1F and the solution was shaken vigorously with chloroform (5 ml). The phases were separated, dithizone in chloroform (0.0001% solution, 5 ml) was added, the mixture was shaken for 1 min in subdued light and the absorbance of the chloroform layer was measured at 500 nm on a Coleman Junior Spectrophotometer. The precision of the method was ±20% at a mercury concentration of 0.1 μg/liter. The recovery of mercury from control samples containing 0.1 μg/liter of mercury was 98%. After the determination of methylmercury, 16 specimens were sent to the FRB Freshwater Institute in Winnipeg where total mercury was determined by flameless atomic absorption spectrophotometry (Uthe et al. 1970).

Results and Discussion

For the 16 samples analyzed for both methylmercury and total mercury, the average fraction of mercury present as methylmercury was 86.0%, with a standard deviation of 42.6. The high value of the standard deviation may be because not exactly duplicate tissue samples were analyzed by both techniques. Total mercury was determined about 2 months after the determination of methylmercury and even though the samples were kept frozen in tightly closed plastic bags, the tissues might have lost a significant amount of water. The average fraction of mercury present as methylmercury is, however, within the range reported in the literature.

Such ill-defined samples are clearly not suitable for the comparison of analytical methods. Homogeneous and stable samples are required for this purpose. Swordfish protein concentrate fulfills these conditions and is therefore a good substrate for the testing of analytical techniques.

The average of five duplicate determinations of methylmercury in a sample of swordfish protein concentrate was 6.32 ppm with a standard deviation of 0.47 ppm. The concentration of total mercury, determined at the FRB Freshwater Institute (L. W. Regier personal communication) was 5.69 ppm. This indicated that within the experimental error practically all mercury in swordfish protein concentrate was present in the form of methylmercury.

The concentration of methylmercury in at least one species of freshwater fish from 8 out of 16 locations exceeded 0.5 ppm (Table 2). These locations were Loch Lomond Lake, Grand Lake, Mactaquac Impoundment, Beechwood Impoundment, Lake Utopia, Digdeguash Lake, St. Patrick's Lake, and Grand Falls.

Some of the mercury-contaminated locations such as Grand Lake, Mactaquac Impoundment, Beechwood Impoundment, and Grand Falls on the St. Croix River are in areas with a variety of industrial activity and receive industrial effluents. Other locations, such as Loch Lomond and Lake Utopia, are in the vicinity of industrialized areas, but do not receive industrial effluents. Finally some locations such as Digdeguash Lake, St. Patrick's Lake, and Chamcook Lake are at appreciable distances from any industrial or agricultural activity. A geological survey report (Abbott MS 1965) lists the occurrence of mercury only on Taylors Island, near Saint John, N.B. Thus the elevated content of methylmercury in fish from the three lakes listed above indicates the possibility of pollution by airborne mercury. The concentration of mercury in precipitation is summarized in Table 3. The amount of mercury deposited from precipitation could be responsible for the levels of methylmercury in fish from lakes not receiving any mercury bearing industrial effluents. For example, the standing crop of eels in several lakes in southeast New Brunswick is 2.6 kg/ha (Smith and Saunders 1955). If all mercury, deposited monthly from precipitation, were accumulated in eels, the concentration of mercury in eels would be 34.7 ppm. This is of course an oversimplification of the situation used to emphasize that an input of minute quantities of mercury may lead to high levels of mercury in the ecosystem, particularly when mercury is retained in it for a long time. It has been estimated that the half-life of methylmercury in several species of fish is from 640 to 1300 days (Jarvenpaa et al. 1970).

The data for Loch Lomond and Chamcook lakes deserve special attention since the lakes are sources of drinking water. Concentration of mercury in water from these lakes was 0.15 and 0.08 μg/liter, respectively. Both values are well below the tentative tolerance limit of 5 μg/liter, proposed for drinking water in the USA (Lesperance 1970).

TABLE 2. Methylmercury in freshwater fishes in New Brunswick.

Species	No. fish (no. samples)	Station	Methylmercury as mercury, *ppm*, wet wt (*range*)	Avg length (*cm*)	Avg wt (*g*)	Food web status
American eel,	6(5)	1	0.07(0.01–0.12)	54.5	317	1st and 2nd
Anguilla rostrata	1	1	0.08	38	114	rank
	8	3	0.75	43		predator
	7	4	0.76	28		
	13(2)	4	0.65(0.55–0.76)	39		
	4	4	2.08	53		
	10	5	0.76	43		
	10(2)	5	0.29(0.24–0.35)	66		
	4	5	0.23	76		
	3	6	0.25	56	209	
	1	7	0.67	87	882	
	1	9	0.60	66	568	
	1	11	0.43	203	1299	
	10(10)	12	0.41(0.19–0.83)	36	109	
	5(3)	16	0.39(0.32–0.48)	59	589	
Museum specimen		16	1.88,1.76[a]	46		
White perch, *Morone*	5	4	1.07	18	92	1st and 2nd
americana	4	5	0.75	20	129	rank
						predator
Yellow perch, *Perca*	1	3	1.05	18	82	1st and 2nd
flavescens	1	6	0.20	16	48	rank
	16(3)	7	0.64(0.47–0.95)	21		predator
Smallmouth bass,	2	4	0.81	13	53	1st rank
Micropterus dolomieui	6(3)	7	1.09(0.84–1.28)	35		predator
	4(2)	8	1.68(1.56–1.80)	46		
	1	11	0.84			
Chain pickerel,	3	3	0.75	39	492	1st rank
Esox niger	1	4	1.58	48	581	predator
	3	6	0.27	48		
	4(4)	11	0.40(0.09–0.60)	40	561	
Brown trout, *Salmo*	2(2)	2	1.57(1.15–1.99)	55	1810	1st rank
trutta						predator
Atlantic salmon,	3	1	0.09	47	Not feeding in fresh water	
Salmo salar	2 Parr, lab		0.20	35	316	2nd rank
	2 Parr, lab		0.12	11	17	predator
Redbreast sunfish,	18(3)	6	0.29(0.15–0.51)			2nd rank
Lepomis auritus						predator
Brook trout,	3	13	0.08	15	41	2nd rank
Salvelinus fontinalis	3	14	0.13	20		predator
	1	15	0.12	15	37	
Pumpkinseed,	3	10	0.19	10	23	2nd rank
Lepomis gibbosus	3(3)	11	0.40(0.33–0.53)	16	85	predator
Fallfish,	3	4	1.63	29		Omnivorous
Semotilus corporalis	2	6	0.27	19		1st rank
	16(8)	11	0.49(0.31–0.84)	24	207	predator
Creek chub, *Semotilus*	5(3)	10	0.36(0.33–0.76)	14	38	Omnivorous
atromaculatus	3(2)	11	0.19	15	70	1st rank
						predator
Brown bullhead,	1	4	0.31	20	85	Omnivorous
Ictalurus nebulosus	1	11	0.59	30		
Golden shiner,	3	5	0.39	12	18	Omnivorous
Notemigonus crysoleucas						
White sucker,	4	1	0.09	22	143	Omnivorous
Catostomus commersoni	1	4	0.37	18	63	
Common shiner,	2	3	0.15	10	14	Omnivorous
Notropis cornutus	2	5	0.37	10		
	1	10	0.21	13	25	
Lake trout,	1	16	1.08,1.04[a]	48		
Salvelinus namaycush						
Museum specimen						

[a]Dry weight basis.

TABLE 3. Concentration of mercury in precipitation.

Date	Type of precipitation	Mercury, μg/liter
	1970	
Oct. 24	Rain	0.13
Nov. 5–6	"	0.13
15–16	"	0.13
21–24	"	0.10
	1971	
Jan. 5–6	Snow	0.20
25–26	Rain mixed with snow	0.15
Feb. 15	Rain	Not detectable
Avg		0.11
Avg deposition, g/ha × month[a]		0.09

[a]Based on average precipitation of 3.42 inches/month.

The Loch Lomond brown trout (Table 2) were probably accumulating mercury by food chain concentration, rather than directly from the water.

The methylmercury concentration in eels from Chamcook Lake indicates elevated levels of mercury in the lake's ecosystem. However, brook trout, held in the laboratory in tap water from Chamcook Lake and fed dry food, had only 0.03 ppm of methylmercury after 2 years. Trout feeding on small Atlantic salmon parr over a period of several months in the laboratory (average weight of salmon eaten, 800 g; C. T. Hatfield personal communication), contained 0.19 ppm of methylmercury. Concentration of methylmercury in salmon parr was 0.12 ppm, which gives a theoretical concentration of 0.31 ppm in the trout (average weight of a trout was 314 g).

Concentrations of total mercury in fish below 0.2 ppm are assumed to be due to naturally occurring environmental mercury (Lofroth MS 1969). Thus methylmercury in fish from the Northwest Miramichi, Waweig, and Digdeguash rivers may be of natural origin. Clarence Lake and Lake Oromocto are on the borderline with slight signs of mercury contamination in the latter (see chain pickerel, 0.27 ppm).

Due to the unequal distribution of mercury in the earth's crust, there cannot be a universal "natural background" concentration of mercury in fish and it is very likely that some locations have much higher backgrounds. Concentrations of methylmercury in the museum specimens (Table 2), when expressed on wet weight basis, assuming that the samples originally contained 70% of water and 10% of ethanol-extractable matter, are 0.36 and 0.21 ppm for eel and lake trout, respectively. Unfortunately, no sample of lake trout was included in the recent sampling. However, the data for Chamcook Lake eels indicate that concentration of methylmercury has changed little, if at all, over a period of at least 46 years.

From the marine species analyzed (Table 4), only Atlantic wolffish, taken in the Deer Island–Campobello Island area, and thorny skate, taken just west of Emerald Bank, contained higher levels of methylmercury. Out of nine analyzed, two wolffish contained 0.56 and 0.61 ppm of methylmercury, whereas the concentration of methylmercury in wolffish taken on the Nova Scotia Banks was 0.09 ppm. In this connection, two significant sources of mercury pollution have been identified in the State of Maine (Anon. 1970a).

The levels of methylmercury in all other marine species analyzed are below 0.13 ppm. The values found agree with those reported from other unpolluted areas (Johnels et al. 1967; Norén and Westöö 1967; Lofroth MS 1969; Ui MS 1969). Atlantic salmon grilse, which feed in the sea, have a lower concentration of methylmercury than Atlantic salmon parr, which feed in fresh water.

The level of methylmercury in an animal is given by the ratio of the rate of uptake to the rate of excretion. From the half-life data (Jarvenpaa et al. 1970) the rate constant of methylmercury excretion is from 0.5×10^{-3} to 1.1×10^{-3} day^{-1}. If the rate of methylmercury uptake is of the same order of magnitude (1×10^{-3} mg/kg × day) the level of methylmercury in the animal will remain constant, or change only slowly. Concentration of methylmercury in the museum specimens indicates that this may be the case in some of the surveyed locations.

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TABLE 4. Methylmercury in marine fish and shellfish from the Bay of Fundy and Nova Scotia Banks.

Station no.	Species	No. fish in sample	Methylmercury as mercury, *ppm*, wet weight
1	Haddock, *Melanogrammus aeglefinus*	5	0.04
2	Pollock, *Pollachius virens*	1	0.04
3	Atlantic halibut, *Hippoglossus hippoglossus*	1	0.03
4	American plaice, *Hippoglossoides platessoides*	5	0.07
5	Witch flounder, *Glyptocephalus cynoglossus*	4	0.03
6	Witch flounder	1	0.04
7	Yellowtail flounder, *Limanda ferruginea*	3	0.04
8	" "	5	Trace
9	" "	5	0.04
10	Winter flounder, *Pseudopleuronectes americanus*	5	0.01
11	Winter flounder	5	Trace
12	Greenland halibut, *Reinhardtius hippoglossoides*	2	0.07
13	Greenland halibut	1	0.05
14	Silver hake, *Merluccius bilinearis*	5	0.05
15	Silver hake	5	0.03
16	Atlantic argentine, *Argentina silus*	5	0.04
17	Atlantic argentine	5	0.03
18	Atlantic herring, *Clupea harengus harengus*	1	0.12
19	Alewife, *Alosa pseudoharengus*	8	0.05
20	Atlantic wolffish, *Anarhichas lupus*	2	0.09
21	Goosefish, *Lophius americanus*	1	0.05
22	Goosefish	1	0.04
23	Thorny skate, *Raja radiata*	3	0.04
24	" "	2	0.24
25	" "	2	0.03
26	" "	3	0.05
27	Longhorn sculpin, *Myoxocephalus octodecemspinosus*	5	0.12
28	" "	5	0.12
29	" "	2	0.05
30	Sea raven, *hemitripterus americanus*	1	0.03
31	Sea raven	2	0.05
32	Spiny dogfish, *squalus acanthias*	1	0.05
33	Lumpfish, *Cyclopterus lumpus*	1	Traces
34	Atlantic eelpout, *Lycodes atlanticus*	2	0.01
35	Ocean pout, *Macrozoarces americanus*	1	0.01
36	Red hake, *Urophycis chuss*	5	0.03
37	Grenadier, *Macrourus* (*Coryphaenoides*) *rupestris*	2	0.05
–	Atlantic cod, *Gadus morhua*	1	0.03
–	White hake, *Urophycis tenuis*	1	0.01
38	Atlantic wolffish, *Anarhichas lupus*	9	0.32
39	Giant scallop, *Placopecten magellanicus*	3	0.03
40	Common rock crab, *Cancer irroratus*	2	0.11
41	Hermit crab, *Pagurus*	1	Trace
42	Purple starfish, *Asterias vulgaris*	1	Trace
43	American lobster, *Homarus americanus*	2	0.11

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An Expression for the Variance of Abundance Estimates Using a Fish Echo Integrator

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MOOSE, P. H., AND J. E. EHRENBERG. 1971. An expression for the variance of abundance estimates using a fish echo integrator. J. Fish. Res. Bd. Canada 28: 1293–1301.

A mathematical model of the fish echo integrator, an acoustic device for measuring the abundance of pelagic fish, is presented. This model is analyzed and general expressions for the mean and variance of the integrator output are obtained. The integrator output can be scaled to provide an estimate of the total number of fish in the volume of water surveyed. The effect of each system parameter on the variance of the estimate is investigated and optimum operational procedures are suggested.

MOOSE, P. H., AND J. E. EHRENBERG. 1971. An expression for the variance of abundance estimates using a fish echo integrator. J. Fish. Res. Bd. Canada 28: 1293–1301.

Nous décrivons un modèle mathématique de l'intégrateur d'échos de poissons, dispositif acoustique capable d'évaluer l'abondance des poissons pélagiques. Nous analysons le modèle et en déduisons les formules générales de la moyenne et de la variance d'émission de l'intégrateur. L'émission de l'intégrateur est étalonnée de façon à fournir un estimé du nombre total de poissons par volume d'eau couvert. Nous étudions l'influence de chaque paramètre du système sur la variance de l'estimé et suggérons une méthode d'opération optimale.

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THE fish echo integrator is an acoustical device for measuring the abundance of pelagic fish stocks. An application of the integrator and comparison of its performance with sample net catches have been reported by Thorne (MS 1970). The purpose of this paper is threefold: (1) to model the system and identify the components of variance; (2) to obtain general expressions for the variance in terms of the parameters of the model; and (3) to suggest operational procedures to minimize the variance and, accordingly, obtain the optimum estimate of a stock size that can be achieved by this technique. The analytical details supporting several of the results given here are contained in reports by Moose (1971) and Ehrenberg (1971a, b). Our goal in this paper is to interpret these analyses and show their practical applicability both for system optimization and for establishing confidence intervals on data produced by the integration method.

In the integration technique, the signal from the echo sounder transducer is used as the input to the integrator system. This system (Fig. 1) consists of a TVG amplifier, a square law detector, a range gate, and an integrator with suitable reset circuitry. The TVG (time-varied-gain) amplifier is used to equalize the signals received from fish at different ranges. The square law detector produces a signal proportional to the squared level of the amplifier signal. The range gate is set by the operator to coincide with a selected depth stratum on the recorder. The time at which the range gate is opened, t_1, corresponds to the depth, d_1; the time at which it is closed, t_2, corresponds to depth, d_2.

The signal from the range gate is electronically integrated in the integrator itself. The integrator output increases during the range gate interval t_1 to t_2, and remains at a constant level (because the input is zero when the range gate is closed) until the gate opens on the next sounding. The integrator continues to accumulate echo data for some selected time (or number of soundings), at which point the value of I is recorded, the integrator is reset to zero, and the process begins again.

The major causes for the variability of I are threefold. First, even though the average density of fish is λ, the actual number in any particular

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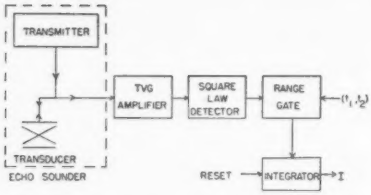

FIG. 1. Schematic block diagram of the integrator.

volume of water may be less than or greater than λ times the volume, V. A common way of describing this randomness is to assume that the number of fish in V obey the Poisson law, i.e., that

$$P(N) = \frac{(\lambda V)^N e^{-\lambda V}}{N!} \quad (1)$$

gives the probability of N fish in the volume V if the average density of fish is λ. If the fish are distributed according to the Poisson law, as we shall assume in this paper, we might expect that the fractional variation would decrease with increasing λ or increasing sample volume, V, or both.

The second source of variability in I is the variation in the amplitude of the echoes from the fish. Echo amplitude will vary with fish size, and fish of the same size will reflect differing amounts of acoustical energy depending upon their aspect angle or even upon details of their individual physical structure (Love 1969).

The third source of variance is associated with the interference that occurs when echoes from two or more fish overlap. Depending upon their relative phase, the echoes may reinforce or reduce the net signal level. In this paper, we will assume that the phases of all the echoes are independent random variables uniformly distributed over the interval of 0–2π. A physical interpretation of this assumption is that the relative ranges of the fish, modulo one wave length, are uniform random variables. The assumption will be made for identical fish seen on separate soundings. For the acoustical wave lengths of most sounders, 1–5 cm, the random phase assumption seems satisfactory.

Other sources of variance that contribute to the variability of I are random ship motion, random noise, and scattering from plankton. All introduce additional components into the actual variance of the integrator signal, but these sources are believed to be second-order effects. They could be included once we obtain a full understanding of the major error sources described above.

Analysis

In this section, the statistical model used in the analysis of the echo integration system is described. The model is used to obtain an expression for the mean and normalized variance of the integrator output.

The analysis begins with a special problem: the integration of returns from a single acoustic pulse. These results are extended to include multiple sounding of both nonoverlapping and overlapping volumes of water. In the analysis, it is assumed that the echo integrator has a TVG that compensates for attenuation of the propagating acoustic wave.

The pressure wave produced by the transducer is assumed to be of the form

$$P_t(t) = K \cos \omega t \, [u(t)-u(t-T_p)], \quad (2)$$

where K is a constant and T_p is the pulse length. The corresponding received pressure wave at time t is

$$P_r(t) = \sum_{m=1}^{N(t)} \frac{P_m e^{-\alpha t}}{t^2} \cos(\omega t+\theta_m) \, [u(t-\tau_m)-u(t-\tau_m-T_p)], \quad (3)$$

where the P_m, identically distributed random variables representing the strength of the signals returned from the fish, are a function of the fish size, aspect angle, position in the beam and species. The θ_m are assumed to be uniformly distributed from 0 to 2π .The random variables θ_m and P_m are assumed to be statistically independent of each other and of $N(t)$. τ_m is the time at which the echo from the mth fish first appears at the transducer. The unit step function, $u(t)$ is defined as

$$u(t) = \begin{cases} 0 & t<0 \\ 1 & t\geq 0 \end{cases}. \quad (4)$$

$N(t)$ is the number of fish producing signals at the transducer through time t. The $\frac{e^{-\alpha t}}{t^2}$ term accounts for the spreading and absorption losses of propagation.

The receiving transducer is assumed to produce a voltage that is directly proportional to the pressure wave. The expression for the transducer voltage after the TVG has compensated for the propagation losses is

$$F(t) \sum_{m=1}^{N(t)} F_m \cos(\omega t+\theta_m) \, [u(t-\tau_m)-u(t-\tau_m-T_p)], \quad (5)$$

where F_m is a constant proportional to P_m and $F(t)$ is a random process that depends upon the signal phases, θ_m; signal amplitudes, F_m; and the number of fish producing returns, $N(t)$. The assumption that the fish are Poisson distributed through the volume implies that $N(t)$ is a nonhomogeneous Poisson process.

$$\text{Prob}(N(t) = k) = \frac{\left(\int_{t_1}^{t_2} \lambda(x)dx\right)^k}{k!} \exp\left[-\int_{t_1}^{t_2} \lambda(x)dx\right]. \quad (6)$$

The expression for the Poisson intensity function $\lambda(t)$ is

$$\lambda(t) = Kt^2 \quad (7)$$

where

$$K = \frac{\pi}{8} \lambda c^3 \sin\left(\frac{\theta_1}{2}\right) \sin\left(\frac{\theta_2}{2}\right),$$

and where c = velocity of sound in water; λ = fish density per unit volume; θ_1 = transducer beam angle in the fore to aft direction; θ_2 = transducer beam angle athwartships. $F(t)$ is a nonhomogeneous filtered Poisson process.

In the echo integrator, the voltage from the transducer is squared and range gated before being integrated. Thus, the output of the integrator for a single transmitted pulse can be represented by

$$I = \int_{t_1}^{t_2} F^2(t)dt, \quad (8)$$

where t_1 and t_2 are the times corresponding to the upper and lower depths of the insonified volume.

The performance of the echo integration system is evaluated in terms of two statistical properties of I: the mean, E_I, and the variance, VAR_I. The relative size of the mean and variance can be calculated by normalizing the variance with E_I^2. Expressions for E_I and VAR_I,

$$E_I = \int_{t_1}^{t_2} E[F^2(\alpha)]d\alpha \quad (9$$

$$VAR_I = \int_{t_1}^{t_2}\int_{t_1}^{t_2} \left\{E[F^2(\alpha)F^2(\beta)] - E[F^2(\alpha)E[F^2(\beta)]\right\} d\alpha d\beta, \quad (10)$$

follow from equation 8 by interchanging the order of integration and expectation.

The required moments of $F^2(\alpha)$ in equations 9 and 10 are evaluated from the joint characteristic function, $\Phi_{F(\alpha),F(\beta)}(u_1, u_2)$, where

$$\Phi_{F(\alpha),F(\beta)}(u_1,u_2) = E[\exp(iu_1F(\alpha)+iu_2F(\beta))]. \quad (11)$$

It follows from the series expansions for the exponential and natural logarithm functions that

$$E[F^2(\alpha)] = -\frac{\partial^2}{\partial u_1^2} \ln \Phi_{F(\alpha),F(\beta)}(u_1,u_2) \mid u_1=u_2=0 \quad (12)$$

and that

$$E[F^2(\alpha)F^2(\beta)] = \frac{\partial^4}{\partial u_1^2 \partial u_2^2} \ln \Phi_{F(\alpha),F(\beta)}(u_1,u_2) \mid u_1=u_2=0 + 2\left[\frac{\partial^2}{\partial u_1 \partial u_2} \ln \Phi_{F(\alpha),F(\beta)}(u_1,u_2) \mid u_1=u_2=0\right]^2. \quad (13)$$

The joint characteristic function for a nonhomogeneous filtered Poisson process, $\Phi_{F(\alpha),F(\beta)}(u_1,u_2)$ is[1]

$$\Phi_{F(\alpha),F(\beta)}(u_1,u_2) = \exp\left\{\int_{t_1}^{\max(\alpha,\beta)} \lambda(\tau)\varphi(\tau,u_1,u_2)d\tau\right\}, \quad (14)$$

where

$$\varphi(\tau,u_1,u_2) = E\left[\exp\left\{iu_1w(\alpha,\tau,F,\theta) + iu_2w(\beta,\tau,F,\theta)\right\}-1\right],$$

and

$$w(\alpha,\tau,F,\theta) = F\cos(\omega\alpha+\theta)[u(\alpha-\tau)-u(\alpha-\tau-T_p)].$$

From equations 12, 13, and 14 it follows that

$$E[F^2(\alpha)] = \frac{E[F^2]}{2} \Gamma(\alpha,\alpha,T_p), \quad (15)$$

and

$$E[F^2(\alpha)F^2(\beta)]-E[F^2(\alpha)]E[F^2(\beta)] = E[F^4]\left\{\frac{1}{4} + \frac{1}{8}\cos\omega(\beta-\alpha)\right\} \Gamma(\alpha,\beta,T_p)$$

[1]The joint characteristic function for a nonhomogeneous filtered Poisson process is given as a problem in Parzen (1962 p. 156).

$$+ \frac{(E[F^2])^2}{2} [\cos \omega(\beta-\alpha)\ \Gamma(\alpha,\beta,T_p)]^2, \quad (16)$$

where

$$\Gamma(\alpha,\beta,T_p) \approx K\ \alpha^2\ [\alpha-\beta+T_p] \text{ for } \begin{cases}\beta>\alpha \\ \beta<\alpha+T_p\end{cases}$$

$$\approx K\ \beta^2\ [\beta-\alpha+T_p] \text{ for } \begin{cases}\alpha>\beta \\ \alpha<\beta+T_p\end{cases}$$

$$\approx 0 \text{ elsewhere.}$$

$E[F^2]$ and $E[F^4]$ are the second and fourth moments of the random amplitude factors F_m.

The evaluation of the integral expressions for E_I and VAR_I can be simplified if typical operating conditions are assumed to satisfy the inequalities

$$t_2-t_1 >> T_p$$

$$t_1 >> T_p$$

$$T_p >> \frac{2\pi}{\omega}.$$

The resulting expressions are

$$E_I = \frac{E[F^2]K\ T_p}{6}[t_2^3-t_1^3], \quad (17)$$

$$VAR_I = \frac{E[F^4]T_p^2K(t_2^5-t_1^5)}{12} + \frac{(E[F^2])^2K^2T_p^2(t_2^5-t_1^5)}{30}. \quad (18)$$

The expression for normalized variance is

$$\frac{VAR_I}{E_I^2} \frac{3E[F^4]}{(E[F^2])^2K(t_2^3-t_1^3)} + \frac{6}{5}\frac{T_p(t_2^5-t_1^5)}{(t_2^3-t_1^3)^2}. \quad (19)$$

The volume of water producing returns at the transducer from t_1 to t_2 is

$$V(t_1,t_2) = \frac{\pi}{24} c^3\ (t_2^3-t_1^3) \sin\left(\frac{\theta_1}{2}\right) \sin\left(\frac{\theta_2}{2}\right)$$

$$= \frac{K}{3\lambda}\ (t_2^3-t_1^3).$$

Solving for K

$$K = \frac{3\lambda V(t_1,t_2)}{(t_2^3-t_1^3)}.$$

Using this expression for K, equations 17 and 18 can be rewritten as

$$E_I = \frac{E[F^2]}{2} V(t_1,t_2)\lambda T_p \quad (20)$$

$$\frac{VAR_I}{E^2} = \frac{E[F^4]}{(E[F^2])^2\ \lambda\ V(t_1,t_2)} + \frac{6T_p\ (t_2^5-t_1^5)}{5(t_2^3-t_1^3)^2}. \quad (21)$$

Since λ is the average number of fish per unit volume, $\lambda V(t_1,t_2)$ is the average number of fish producing returns from the volume insonified. $E[F^2]\ T_p/2$ is the expected return from a single fish. If $E[F^2]\ T_p/2$ can be determined, the output of the integrator can be scaled to provide an estimate of the fish density.

The first term in the normalized variance, equation 21, decreases as the inverse of the expected number of fish. The second term is independent of the fish density and is directly proportional to the pulse length, T_p.

The results for a single transmit pulse obtained above can now be generalized to include integration of the returns from multiple soundings of both nonoverlapping and overlapping volumes. Define I_T as the integrator output after the integration of the returns from N pulses. Then

$$I_T = I_1+I_2+\cdots+I_j+\cdots I_N, \quad (22)$$

where I_j is the integral of the returns from the jth pulse. If it is assumed that the fish density, λ, is the same for all N pulses and that each pulse is range gated to the same depth interval, it follows that

$$E[I_T] = NE_I, \quad (23)$$

where E_I is given by equation 20. If a different volume of water is sampled with each pulse, the individual components of I_T are independent and the variance of I_T is

$$VAR[I_T] = N\ VAR_I, \quad (24)$$

where VAR_I is given by equation 18. The normalized variance for I_T follows directly from equations 23 and 24,

$$\frac{VAR[I_T]}{(E[I_T])^2} = \frac{VAR_I}{N\ E_I}. \quad (25)$$

The expression for $VAR[I_T]$ is more difficult to obtain when there is overlap of the volumes of water insonified by adjacent acoustic pulses. For this analysis, it is convenient to consider the depth stratum d_1 to d_2 as several layers. The initial and

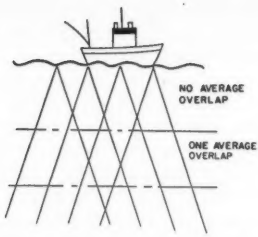

FIG. 2. Division of depth strata into layers with an integer amount of overlap.

final depths of each layer are determined such that the following two conditions are satisfied: the average number of times each layer is insonified is an integer, and the depth of each layer is large compared to $cT_p/2$. This last condition insures that the integrator outputs for adjacent layers are approximately independent. A typical example of this division is shown in Fig. 2. The total variance is approximated by the sum of the variances for each layer.

$$\mathrm{VAR}(I_T) = \sum_i N_i \mathrm{VAR}[I(i)], \tag{26}$$

where VAR[I(*i*)] is the variance of the integrated returns from an insonified volume in the *i*th layer, and N_i is the number of distinct insonified volumes in the *i*th layer.

$$N_i = \frac{N}{n_i}, \tag{27}$$

where N is the total number of acoustic pulses and n_i is the average number of countings for an insonified volume in the *i*th layer. If F_{ij} (*t*) is defined as the signal returned from a volume in the *i*th layer insonified by the *j*th acoustic pulse, and if I_{ij} is the corresponding integrator output, then

$$I_{ij} = \int_{t_{i-1}}^{t_i} F_{ij}^2(t)\,dt \tag{28}$$

and

$$\mathrm{VAR}[I(i)] = \sum_{j=1}^{n_i} E[I_{ij}^2] + \sum_{\substack{j=1 \\ l\neq j}}^{n_i} \sum_{l=1}^{n_i} E[I_{ij}I_{il}] - \sum_{j=1}^{n_i} \{E[I_{ij}]\}^2 - \sum_{\substack{j=1 \\ l\neq j}}^{n_i} \sum_{l=1}^{n_i} E[I_{ij}]E[I_{il}]. \tag{29}$$

For typical conditions, it is reasonable to assume that the same fish are present in any given volume over a period of several transmission pulses. However, in general, the position of a fish in relation to the transducer changes enough from pulse to pulse to cause the phase of signals from the same fish and different pulses to be uncorrelated. For this condition then, VAR[I(*i*)] obtained from equations 12, 13, 14, 28, and 29 is

$$\mathrm{VAR}[I(i)] = \left[\sum_{j=1}^{n_i} E[F_{ij}^4] + \sum_{\substack{j=1 \\ j\neq l}}^{n_i} \sum_{l=1}^{n_i} E[F_{ij}^2 F_{il}^2]\right] \cdot \left[\frac{KT_p^2(t_i^3 - t_{i-1}^3)}{12}\right] + \sum_{j=1}^{n_i} \frac{\{E[F_{ij}^2]\}^2 K^2 T_p^3 (t_i^5 - t_{i-1}^5)}{30}, \tag{30}$$

where $E[F_{ij}^2 F_{il}^2]$ is the joint second moment of the random amplitude factor for the *j*th and *l*th insonification of the *i*th layer. $E[F_{ij}^2\, F_{il}^2]$ is a function of the aspect angle and position of the fish in the beam pattern during the *j*th and *l*th pulse. $E[F_{ij}^2\, F_{il}^2]$ is a minimum when

$$E[F_{ij}^2 F_{il}^2] = E[F_{ij}^2]\, E[F_{il}^2] = (E[F^2])^2$$

and is a maximum when

$$E[F_{ij}^2 F_{il}^2] = E[F^4].$$

The effect of the correlation between F_{ij}^2 and F_{il}^2 is conveniently included in the variance expression in terms of a correlation coefficient ρ_{jl} (*i*), where

$$\rho_{jl}(i) = \frac{E[F_{ij}^2 F_{il}^2] - (E[F^2])^2}{E[F^4] - (E[F^2])^2}. \tag{31}$$

Using this definition for ρ_{jl} (*i*), the expression for VAR[I(*i*)] is

$$\mathrm{VAR}[I(i)] = \left[(n_i + C_{n_i})E[F^4] + (n_i^2 - n_i - C_{n_i})(E[F^2])^2\right] \left[\frac{KT_p^2(t_i^3 - t_{i-1}^3)}{12}\right] + \frac{n_i (E[F^2])^2 K^2 T_p^3 (t_i^5 - t_{i-1}^5)}{30},$$

where

$$C_{n_i} = \sum_{\substack{j=1 \\ j \neq l}}^{n_i} \sum_{l=1}^{n_i} \rho_{jl}(i). \tag{32}$$

The normalized variance of the estimated fish density after the integration of N pulses is determined from equations 19, 23, 26, 27, and 32.

$$\frac{\mathrm{VAR}[I_T]}{\{E[I_T]\}^2} = \frac{1}{N}\frac{\mathrm{VAR}_I}{E_I^2} + \frac{3}{NK}\sum_i \left[\frac{E[F^4]}{(E[F^2])^2}\frac{C^{ni}}{n_i} + n_i - \frac{C_{n_i}}{n_i} - 1\right]\frac{[t_i^3 - t_{i-1}^3]}{[t_2^3 - t_1^3]^2}. \tag{33}$$

When the volumes insonified do not overlap, $C_{n_i} = 0$, $n_i = 1$, and equation 33 reduces to 25.

Applications of the Variance Expression

An investigation of the relation between various system parameters and the normalized variance was carried out with the aid of a digital computer. A program was written to numerically evaluate equation 33 and plot the normalized variance as a function of fish density for a particular set of system operation conditions.[2] Some of the more important results of this investigation are illustrated by the following examples.

The integration of the returns from a single acoustic pulse shows that the normalized variance as a function of fish density can be approximated by two straight line segments (Fig. 3A). The portion of the curve with the 45° slope is determined by the first term in the single pulse normalized variance expression (equation 21). This term is inversely proportional to the expected number of fish in the insonified volume. The horizontal portion of the curve is determined by the second term in equation 21, which is proportional to the pulse length, T_p.

An increase in the beam width of the transducer results in an increase in the insonified volume and a corresponding decrease in the first term of equation 21. The second term in equation 21 is not a function of the beam width and dominates for high fish densities (Fig. 3B). Changes in the pulse length affect the performance of the system when estimating fish populations with high densities (Fig. 3C).

The expression for normalized variance for multiple pulses can be split into two parts — just as it was for the single pulse expression. Again, one term is inversely proportional to the fish density and the other term proportional to the pulse length. The variance per pulse is minimized by increasing the distance traveled between pulses (Fig. 3D). Increasing the distance between pulses is one way of reducing the amount of beam overlap.

On the basis of the foregoing, it should not be assumed that the best operational procedure is to minimize the amount of beam overlap. Beam overlap is desirable when the total distance traversed by the boat is fixed (Fig. 3E). The actual form of the correlation coefficient, $\rho_{jl}(i)$, is not known and can be determined only experimentally. The effect of this parameter on the normalized variance can be shown by considering its two extreme values, $\rho_{jl}(i) = 0$ and $\rho_{jl}(i) = 1$ (Fig. 3F). The sensitivity of the normalized variance to the form of $\rho_{jl}(i)$ increases when the number of multiple countings increases. However, the close spacing of the curves in Fig. 3F shows that the accuracy of the variance expression is affected little by the accuracy of the determination of the form of $\rho_{jl}(i)$.

Discussion

This study has shown that the mean value of the output of an echo integrator is directly proportional to the product of the average fish target strength, $E(F^2)$, and the average density of fish λ, equation 20, and that the normalized variance consists of a term that is inversely proportional to the total number of fish insonified plus a term proportional to pulse length (equations 21 and 33).

The density function of the integrator output may be assumed to be normal since it is composed of the superposition of many independent random variables. The mean and variance are therefore sufficient statistics to determine levels of confidence for specified intervals of accuracy.

If multiple overlapping soundings are used, the variance depends on the ping-to-ping correlation, $\rho_{jl}(i)$, of the reflection coefficients of the fish. The variance is reduced if the echo strengths on sequential pings are independent random variables, ($\rho_{jl}(i) = 0$, $|j-l| > 0$). Just how rapidly $\rho_{jl}(i)$ falls off with increasing $|j-l|$ is not known; however, we believe that the falloff will be more rapid at higher frequencies where the echo strength varies more rapidly with small aspect changes. The contrast between fully correlated and fully uncorrelated amplitudes appears small for practical cases (Fig. 3F).

The analysis shows that the variance can be reduced by decreasing the pulse length and by increasing the insonified volume. Both of these

[2]A program listing and description of the numerical technique used to evaluate equation 33 is contained in Ehrenberg (1971b).

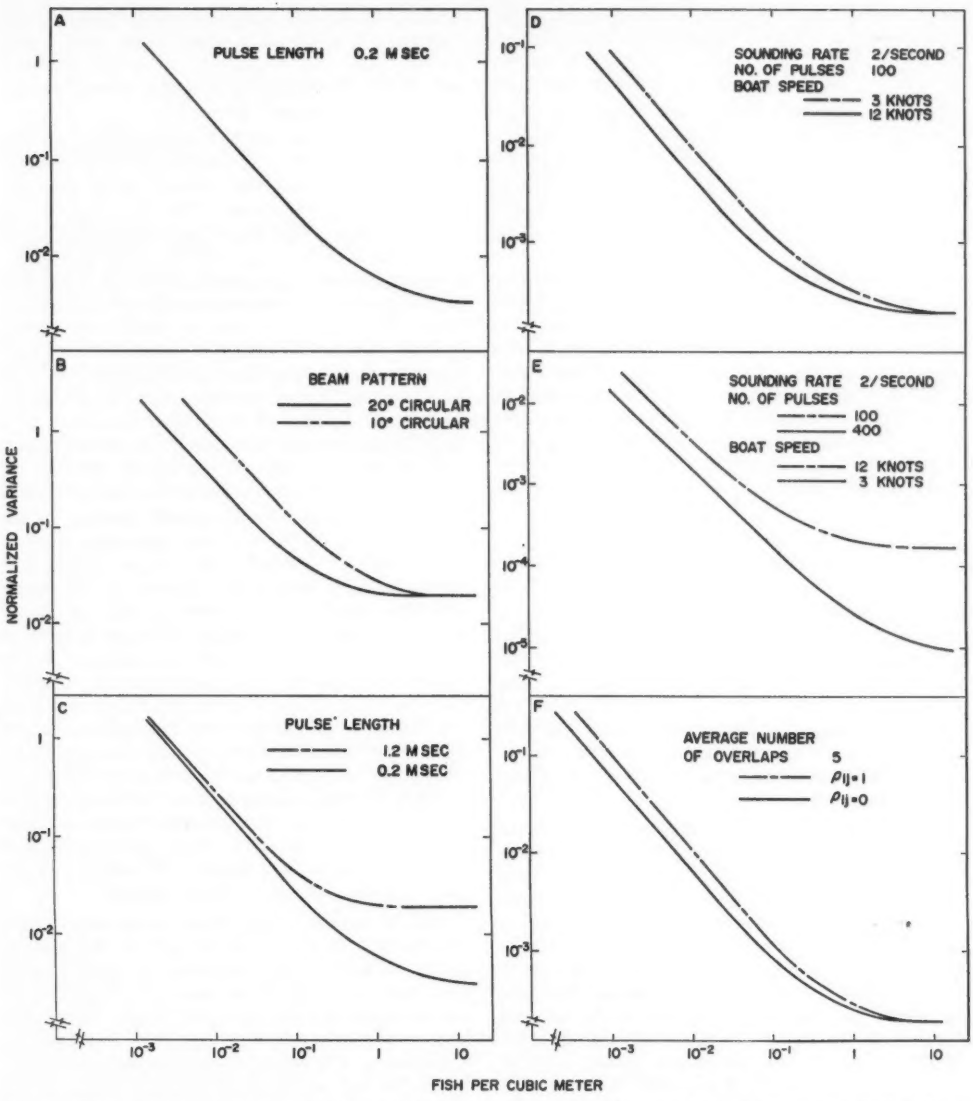

FIG. 3. Normalized variance as a function of fish density. Unless otherwise stated, depth interval is 10–40 m, beam pattern is 20° circular, TVG is 20 log R, and pulse length is 1.2 msec.

conclusions are subject to conditions implicit in certain assumptions that have been made. As the pulse length is made shorter, the receiver bandwidth must be broadened to accomodate the short echoes from single fish. However, this admits more noise and the point will be reached where it cannot be neglected. Actually, there is a strong motivation to keep the pulse length as long as possible in order to minimize the receiver bandwidth. This has two advantages. First, there will be a significant increase in the signal-to-noise ratio. There will be a reduction in the noise level, since the receiver bandwidth can

be narrowed, and there will be an increase in the signal level, equation 17. Second, the narrowband data can be recorded more efficiently than the broadband data. For example, a digital recording of the data can have sample values spaced in direct proportion to the pulse length. If the integrator system is implemented digitally (either in realtime or in postsurvey analysis), the amount of processing time will be in direct proportion to the signal bandwidth and may therefore be reduced by increasing the pulse length.

It is clear from the results of the last section that for every fish density, reducing the pulse length beyond a certain critical value has an insignificant effect on the system performance. Although further shortening of the pulse length may bring about small theoretical improvements, any improvement will probably be offset by other factors such as noise. One should shorten the pulse length, if possible, to the critical value. This value could be based on the criteria

$$\frac{6}{5}\frac{T_p}{N}\frac{(t_2^5-t_1^5)}{(t_2^3-t_1^3)} \leq \epsilon_{T_p},$$

where ϵ_{T_p} would be considered an acceptable contribution to the variance due to this source. For example, if $t_1 = 10$ msec, $t_2 = 20$ msec ($d_1 = 7.5$ m, $d_2 = 15$ m), and we sound for 20 times, a pulse length of 1 msec will cause a variance of only 0.5% in the estimate of λ. The corresponding standard deviation of 7% in its distribution may well be acceptable when considered in concert with the other estimation errors.

Insonifying as large a volume as possible to reduce the variance (equation 21) agrees with our understanding of sample statistics in general, i.e., that more independent observations improve the quality of the estimate. However, this observation is intimately related to the assumption of homogeneity of the true population density throughout the insonified volume. Here, increasing both the length of the range gate as well as the angular coverage of the transducer potentially admits more echoes. In reality, the fish are in shoals, schools, or layers in which, to first order at least, their density may be considered uniform. The geometry of the sound beam must be constrained to insonify these aggregations for the preceding analysis to be valid. This is also true for the problems of roll, pitch, and heave as well as transducer side lobes (and nonideal beam patterns in general). So long as the stock under observation spans the volumes that are sampled, maximizing the sample volumes will improve the estimate. However, admitting signals from regions that are not occupied by the stock is detrimental: with respect to noise, the estimate will be biased upward; in the case of ship motion, when the beam is being partially displaced out of the layer on some of the soundings, the estimate will be biased downward.

When multiple soundings and beam overlap are considered, we find that increasing the number of soundings along a given transect never increases the variance of the estimate. Thus no harm accrues from sounding as rapidly as possible (barring second echo interference), but, as with pulse length reduction, a point of diminishing returns is reached when little new information is gathered from more pings. Thus, for recording or digital processing, or both, one would minimize the amount of data by using as few soundings as necessary to achieve an acceptable level of variance. As a rule of thumb, one should attempt to insonify each fish at least once. Thus, the ping rate should be commensurate with the speed of ship travel and the width of the transducer pattern at the top of the depth stratum.

Both the fourth and the second moment of the fish reflection coefficients play important roles in the expressions derived in this paper. The second moment, $E(F^2)$ (commonly known as the target strength), must be determined for the population being surveyed if the integrator output is used to obtain an absolute estimate of density. If the relative abundance of two populations is required, then it is only necessary to assure that the two have the same size distribution and are assessed during similar periods of activity. It may be possible to obtain absolute estimates by estimating the target strength of single targets in situ. Such a procedure will compensate for the downward bias of target strength due to nonideal beam patterns as well as provide the correct factor with which to scale the integrator voltage to obtain density.

For the variance expressions, equations 21 and 33, the ratio of the fourth moment to the squared second moment of the reflectivity is required. This ratio takes on a minimum value of 1 if all the F's are constants but can be much larger, e.g., 6 for an exponential distribution and 2 for the Rayleigh distribution. The variation in reflection coefficient is due to size variation from fish to fish, aspect variations, and variable location in the beam pattern. Additional theoretical and experimental work is needed to identify the probable range of values for this ratio and its variation with length to wavelength ratio as well as species and stock composition. Other problems to be considered are pulse stretching and distortion, shadowing of adjacent fish, and multiple reflections in dense aggregations. The fundamental assumption that the fish are Poisson-distributed needs additional

consideration, particularly for the case of schooled fish.

Acknowledgments

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Effects of Oxygen Depletion and of Carbon Dioxide Buildup on the Photic Behavior of the Walleye (*Stizostedion vitreum vitreum*)

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SCHERER, E. 1971. Effects of oxygen depletion and of carbon dioxide buildup on the photic behavior of the walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Bd. Canada 28: 1303–1307.

In laboratory experiments under overhead white light, walleyes (*Stizostedion vitreum vitreum* (Mitchill)) displayed a negative phototactic response consistent with field observations and histological features of the walleye eye. The strength of this innate response proved to be largely dependent on the given respiratory situation: during oxygen depletion and during buildup of free carbon dioxide, this response diminished to the point of obliteration, which occurred at levels leaving mobility and equilibrium of the test specimens still intact. Whereas during the course of oxygen depletion a wide indifference zone (little or no change of light behavior) could be observed from saturation (= acclimation) level down to about 4–2 mg/liter, the fish reacted rather sensitively to increase of free carbon dioxide. Starting from acclimation levels of 1.0–2.0 mm Hg CO_2, the light avoidance response declined gradually while approaching tensions of 5 mm Hg or more, being completely abandoned at levels above 15 mm Hg. This effect could not be attributed to *p*H shifts.

SCHERER, E. 1971. Effects of oxygen depletion and of carbon dioxide buildup on the photic behavior of the walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Bd. Canada 28: 1303–1307.

Au cours d'expériences en laboratoire sous éclairage vertical blanc, les dorés (*Stizostedion vitreum vitreum* (Mitchill)) ont exhibé un phototactisme négatif conforme aux observations en nature et aux caractéristiques histologiques de l'oeil du doré. L'intensité de la réaction innée dépend à un haut degré de l'état respiratoire: lorsqu'il y a épuisement d'oxygène et accumulation de CO_2 libre, la réaction diminue jusqu'au point d'extinction. Ceci se produit à des niveaux qui n'affectent pas encore la mobilité et l'équilibre des sujets d'expérience. En période d'épuisement d'oxygène on a observé des zones sans changement de phototactisme, soit à partir du point de saturation (= acclimatation) jusqu'à des teneurs d'environ 4–2 mg/liter. Par contre, les poissons sont plutôt sensibles à l'augmentation de la teneur en CO_2 libre. A partir de niveaux d'acclimatation de 1.0–2.0 mm de Hg CO_2, le phototactisme négatif diminue graduellement aux approches de 5 mm de Hg et disparait complètement à des niveaux supérieurs à 15 mm de Hg. On ne peut attribuer ces réactions aux déplacements du *p*H.

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AVOIDANCE of high light intensities is reported to contribute strongly to the spatial distribution of the walleye (*Stizostedion vitreum vitreum* (Mitchill)) (Regier et al. 1969; R. A. Ryder personal communication). That account, based upon field observations, is in accordance with a striking feature of the walleye retina, which provoked the common name of this species: a reflecting tapetum lucidum favoring perception at low levels of light intensity (Wunder 1930; Moore 1944; Ali and Anctil 1968).

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Hence, the walleye is said to be negatively "phototropic" or, more properly, phototactic. It is known, however, from many species throughout the animal kingdom, that responses toward light quite commonly are subject to modification, related to different configurations of internal and environmental conditions.

The lack of experimental data on photic responses of *Stizostedion* prompted the present studies. Effects of respiratory stress were chosen for investigation because of the general physiological and ecological relevance of O_2 and CO_2 conditions, and because these environmental parameters are

particularly susceptible to numerous forms of pollution.

The aim was to determine whether the walleye's photic response indeed is influenced by sublethal changes of O_2 and CO_2 conditions, and, if so, in what way and to what extent.

Materials and Methods

Second summer walleyes were used in all experiments. They were hatched by the provincial hatchery at Swan Creek/Lake Manitoba, reared in one of the Freshwater Institute's experimental fish farming lakes south of Riding Mountain National Park, and held for more than a year in seemingly good condition, with no disease-caused mortalities, fed on frozen brine shrimp. They were acclimated for at least 4 weeks to 22 C, their reported optimal summer temperature (Ferguson 1958). Experiments were carried out between July and October.

The test aquarium (40 × 45 × 90 cm) was equipped with an overhead illumination panel, consisting of six General Electric "Chroma" fluorescent bulbs, color rendering index R_a92+, three of the type 55 (5500°K), and three of type 70 (7000°K), fixed in alternating order and parallel. The voltage was regulated to ±0.1% on line and load. A sheet of white plexiglas (6 mm thickness) was mounted beneath the bulbs as a diffusing screen. Illumination was measured with a Lange Luxmeter, and a Clairex photoconductive cell.

The test water was circulated through an adjacently connected mixing cylinder, where make-up water (replacement rate 99% in 28 hr), air, CO_2, and N_2 were added. Gas flow was regulated by hand with the help of pressure gauges. There was a feed-in tube in each corner of the aquarium, with 42 boreholes over the entire depth of the water column to obtain a homogeneous distribution of the gas-manipulated water; back-flow was allowed through a perforated Plexiglas tube in the center, leading through the rear wallplate. Determinations of free CO_2 were carried out titrimetrically and converted to tensions (Fry 1957) employing the usual calculations based upon 760 mm Hg normal pressure. To convert tension values approximately into milligrams per liter multiply by 2.15. O_2 was measured with a YSI oxygen meter model 54, *p*H with Radiometer model PHM 53.

For each experiment, three random-sampled fish were used. They were kept at a LD 12:12 cycle (7:00 AM–7:00 PM) and fed daily ad libitum between 8:30 AM and 9:00 AM. All 27 experiments started early in the afternoon.

Two experimental set-ups were employed. (1) A shelter was offered, a rectangular plate (11 × 13 cm) covered by black polyethylene, suspended 3.5 cm above the bottom (enough to avoid a thigmotactic reaction). After a series of preliminary tests, only six experiments were conducted with this shelter arrangement (reasons given in Results following). (2) The front plate of the aquarium was marked with 13 parallel horizontal lines at 3-cm intervals, providing 14 distinguishable layers from bottom to surface, corresponding to a range of light intensities from 700 to 2400 lux.

The fish were observed through a one-way mirror covering the frontplate, and their vertical positions manually recorded every 10 sec. If a walleye was overlapping two, that layer was counted that harbored the greater part of the body. Arithmetic means of the resulting 18 position values per minute were plotted.

Results

Response to a Light Shelter

Under the given light conditions, the walleyes occupied the shelter area as long as CO_2 tensions were kept below 5 mm Hg and O_2 above 5 mg/liter. Occasionally, brief excursions into the unshaded space took place. These lasted only a few seconds and rarely longer than half a minute, with a frequency of one to three times per individual per hour. It took 150–200 min to deplete the oxygen to 1 mg/liter, 80–120 to reach 12 mm Hg CO_2.

Changing O_2 or CO_2 conditions caused the following locomotor reactions:

Phase	Fish reaction	mg/liter O_2 or	mm Hg CO_2
1	None	Above 6	1.5–2.0
2	Increase of mobility, but still staying under shelter	6–5.5	2.0–5.0
3	Further increases of mobility, sometimes darting out, but returning within seconds; one or two individuals may stay outside with some portion of the body, head or tail	5.5–4	5.0–8.0
4	Rapid increase of darting-out reactions, one or two individuals staying outside for more than 1 min	4–2	8.0–12
5	All three specimens staying outside, increasingly moving upward	2–1	Above 12

The mode and sequence of reactions appeared to be the same for O_2 depletion and CO_2 buildup. This shelter arrangement was quite suitable to demonstrate the change of a position normally maintained under the guidance of a preference/avoidance mechanism. However, since the shaded area was restricted, any considerable increase in locomotor activity would, of course, result in a fish leaving the shelter. Also, it was not possible to quantitatively follow in sufficiently small steps a graded photic reaction of the fish that might go

along with the gradual change of conditions. Indeed, the observed intentional movements, darting out or staying out with part of the body, pointed to a continuous change in the behavioral state of the fishes before finally giving up the shelter.

Thus, it was desirable to use another experimental device that would allow continuous quantitative recordings of changing behavior.

Response to Overhead Light

When half of the aquarium was shielded by a black overhead sheet, the walleyes exhibited a strong preference for the darkened part. Moreover, in the dark half they moved freely from the bottom to the surface, as correspondingly they did in total darkness, observed by an infrared viewing apparatus. If they entered the illuminated half, they would head for the bottom.

These observations suggested the use of vertical distribution, i.e., the fishes' distance from the overhead light, as a criterion for the strength of their light avoidance.

Reaction to O_2 depletion

During the process of O_2 depletion, a wide range, from near saturation level at 8.5 mg/liter to 1.5 mg/liter, with little or no reaction, was observed (Fig. 1). When 1.5–1.0 mg/liter oxygen was reached, movements toward the upper half of the aquarium occurred. Preliminary experiments had shown that the extension of the depletion procedure to less than 0.6 mg/liter would lead to a loss of coordination and equilibrium. Therefore, depletion was stopped at 1 mg/liter; at that point, oxygen was added, whereupon the fish returned to lower levels.

Reaction to CO_2 buildup

Quite distinct from the reaction to oxygen depletion, there was no large indifference zone between the level of acclimation and reaction threshold when increasing the CO_2 tension. Upward movements were observed between 3 and 10 mm Hg CO_2. Complete extinction of the light avoidance response was caused by 14–18 mm Hg (about 30–40 mg/liter). The fish kept moving to the uppermost layers and stayed there, swimming around until the free CO_2 level was lowered again (Fig. 2, 3). Throughout these experiments, the oxygen content never dropped below 7.6 mg/liter.

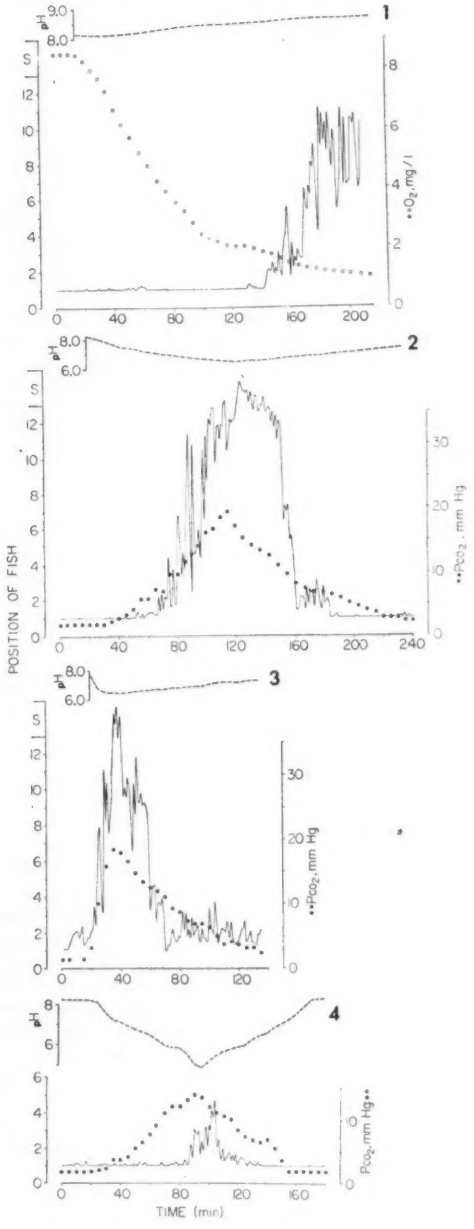

FIG. 1–4. Reactions of walleyes to O_2 depletion, changing CO_2 tension, and changing *p*H, under equal conditions of overhead light; individual experiments. Lefthand ordinates denote mean vertical positions of three test specimens plotted in 1-min intervals. S = upper level to water surface. Righthand ordinates: oxygen contents (open circles) and carbon dioxide tensions (solid circles).

The method of gas manipulation used did not allow exactly predetermined gas curves. A comparison of Fig. 2 and 3 (the latter representing a rather extreme case) shows how closely the reactions followed different slopes of the CO_2 curve.

Reaction to pH change

Both O_2 and CO_2 manipulations were, of course, accompanied by *p*H changes. To evaluate the possible role of hydrogen ion concentration, HCl and NaOH were administered in such a manner as to duplicate the *p*H slopes incurred in the O_2 and CO_2 experiments. There was no reaction at all when raising the *p*H from 8 to 9, using NaOH. An upward movement of the fish could only be seen by dropping the *p*H to less than 6, which resulted in the liberation of free CO_2 up to 14 mm Hg. If we compare this with the reaction to CO_2 addition (Fig. 2, 3), the upward movement appears to be rather suppressed (Fig. 4). Moreover, the reaction of fish was observed as well at rising (O_2 depletion) or falling (CO_2 addition) values of *p*H. Thus, I found no indication that *p*H of the water per se might play a causative role.

FURTHER OBSERVATIONS

Several symptoms of respiratory distress in fish have been frequently described: "yawning," "coughing," increase of ventilation, increase of activity (e.g., Höglund and Härdig 1969; Höglund 1961). All these signs could be observed with our walleyes, too. As mentioned, the increase of activity was one of the reasons for not continuing the use of a restricted shaded area. An increase in locomotor activity when caused by means other than O_2/CO_2 manipulation (e.g., disturbance by an observer when the one-way mirror was removed, or by tapping on the aquarium) would take place horizontally, in the lower layers of the aquarium.

Also observed were changes of coloration, ranging from the usual dark yellow or greenish ground color with dark grey to black spots and bars to a rather homogeneous paleness. However, no consistency could be established along O_2 or CO_2 slopes in the present experiments. Further studies may clarify whether the pineal organ plays a role both in phototaxis and integumental pigment reaction.

Discussion and Conclusions

Innate behavioral responses to environmental factors, such as a negative phototaxis, can be considered as essential tools developed by the species along with the evolutionary process of establishing its ecological niche. By means of preference and avoidance mechanisms, environmental identities become directive clues that guide the animal in selecting suitable factor configurations.

Deterioration of respiratory conditions leads to diminution or temporary abolition of an avoidance response that normally largely determines the distribution of the walleye within its environment. This reaction occurs well before incipient lethal conditions are reached, as judged by the undisturbed coordination of movements and equilibrium of the test specimens, and return to their usual photic behavior immediately after cessation of stress.

There appears to exist a relatively large indifference zone with regard to oxygen reduction. Compared with this, increases of free CO_2 lead to rapid and more vigorous reactions. One could think of well-known effects: the oxygen utilization being hampered by CO_2 uptake into the blood (Root 1931; Black et al. 1954). However, it is still uncertain how changing O_2/CO_2 conditions are perceived by fishes (Höglund and Härdig 1969; Konishi et al. 1969; Powers and Clark 1942), and hence what the pathway might be from stimulus to response.

A more comprehensive quantification of the effects may be obtained by thorough analysis of adaptation and acclimation processes, using more elaborate methods of gas manipulation. From the present experiments one can safely conclude that all attempts to establish thresholds for photic behavioral responses of this species have to be done under clearly defined respiratory conditions. The CO_2 pressure should be given particular attention.

In field situations, decomposition of organic material (deposits on the lake bottom) or even short-term discharges of acidifying wastes (liberating free CO_2 out of the bicarbonate system) might cause significant changes of the commonly observed walleye distribution, such as staying at the lake bottom during bright noon hours.

Further investigations should regard the possibility of temperature-related seasonal modifications. Hart (1957), using three different species, found a higher CO_2 sensitivity at low temperatures, as measured by the CO_2 pressures required to asphyxiate the fish.

The light avoidance response obviously is not a rigid mechanism determining the distribution regardless of other factors. If it were, it would not allow the fish to escape detrimental situations as long as it is able to. This, it seems to me, is the biologically essential aspect of abandoning a reflex that would become destructive if maintained under adverse conditions.

Acknowledgments

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A Reconsideration of Aspects of Growth, Reproduction, and Behavior of the White Whale (*Delphinapterus leucas*), with Reference to the Cumberland Sound, Baffin Island, Population

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BRODIE, P. F. 1971. A reconsideration of aspects of growth, reproduction, and behavior of the white whale (*Delphinapterus leucas*), with reference to the Cumberland Sound, Baffin Island, population. J. Fish. Res. Bd. Canada 28: 1309–1318.

The beluga or white whale, *Delphinapterus leucas* (Pallas), was studied in Cumberland Sound, Baffin Island. Layering in teeth and mandibles plus body size were used to determine age. Sexual maturity is attained at 5 years for females and at 8 years for males, with potential life span estimated to be 30 years. Whitening of the skin begins after 6 years.

Multiple ovulations and accessory corpora lutea are typical of beluga. The breeding season is in May, and after 14.5 months gestation single births occur in late July or early August. Lactation lasts about 2 years, resulting in a 3-year reproductive cycle. Tooth eruption begins late in the 2nd year with partial eruption by the 3rd. This population appears to have been overexploited by commercial hunting. Productivity estimates for this species are 43% of those implied in previous studies.

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L'auteur a étudié le béluga ou baleine blanche, *Delphinapterus leucas* (Pallas), du détroit de Cumberland, île de Baffin. Les couches dentaires et les mandibles, en plus de la taille de l'animal, ont servi à déterminer l'âge des bélugas. Les femelles atteignent leur maturité sexuelle à l'âge de 5 ans, et les mâles à 8 ans. Le béluga peut vivre jusqu'à environ 30 ans. La peau commence à blanchir après la 6ième année.

Les bélugas sont caractérisés par des ovulations multiples et des corps jaunes accessoires. L'accouplement a lieu en mai et la mise bas, après une période de gestation de 14.5 mois, d'un seul baleineau a lieu fin de juillet, début d'août. La période de lactation est d'environ 2 ans, d'où un cycle de reproduction de 3 ans. La percée des dents commence à la fin de la 2ième année et n'est pas encore complétée à la fin de la 3ième année. La population sous étude semble avoir été surexploitée commercialement. La productivité de l'espèce est estimée à 43% de celle implicite dans des études antérieures.

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BETWEEN 1966 and 1969 a study was made of white whales, or beluga, in Cumberland Sound, Baffin Island. All animals captured were utilized by the local inhabitants. Indexes of growth and reproduction were observed, with the object of contributing information on population size and productivity. Particular attention was paid to the association of animals captured in passive nets. Results of the comprehensive study by Kleinenberg et al. (1964) and less extensive studies by others are reassessed in terms of the present findings.

Methods

Of the total 124 animals, 107 (86%) were captured in nets and the remainder hunted. All were captured while entering or within Clearwater Fiord, at the head of Cumberland Sound, during mid-July to mid-September. Most of the nets were set in shallow areas of the fiord, and thus there was a bias (evident in Fig. 1) toward females with newborn, which apparently favor shallow waters.

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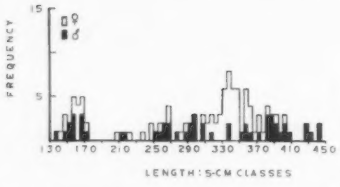

FIG. 1. Length frequencies in 5-cm classes for total sample of 124 beluga, illustrating the bias toward adult females with newborn.

Calves were usually enmeshed immediately beside adult females. All specimens were marked according to their proximity to others before removal. Specimens were towed ashore and examined at low tide.

Skin color, superficial marks, and scars were recorded. Total length was measured in a straight line from the tip of the upper lip to the base of the tail notch. Maximum girths were measured as the full girth on small animals that could be easily moved and as half the girth on larger animals (middorsal and midventral lines are apparent at maximum girth).

Small animals only were weighed intact on a spring scale. Fat thickness was measured dorsally, laterally, and ventrally at maximum girth. Extent and number of erupted teeth of all young specimens were recorded. The distal half of one mandible containing several teeth was collected for age determination. Initially, both testes were removed and measured along three axes. Later, only one testis per individual was measured, though both were checked for anomalous structures. Median sections were collected from testes and epididymis and preserved in formalin and Bouin's fluid.

Females were examined for external milk secretion. Length, width, and depth of mammary tissue was measured during the skinning operation. The complete reproductive tract was opened and examined. Internal collapsed diameters were taken at the midpoint of each cornus. Fetuses were measured and sexed. Recording in the field the position (left or right) and approximate size of apparent corpora lutea, corpora albicantia, and follicles aided in orientation of ovaries during laboratory analysis. Ovaries were hand-sliced to a thickness of 2–3 mm and corpora diameters measured in three axes to establish the mean diameter.

Testes volumes were calculated from measurements taken in three axes, considering the testis to be cylindrical with hemispherical ends. Tissue was imbedded in wax, sectioned and stained, and examined microscopically.

Teeth were sectioned lengthwise as half-sections and examined under a dissecting microscope. Mandible sections were imbedded in bio-plastic, sectioned and stained, and examined microscopically.

Results

It is most probable that two tooth layers and one mandible layer are deposited annually. Based on these criteria, plus body growth increments of young animals, the growth rate of beluga is considerably slower than proposed by Kleinenberg et al. (1964).

Breeding and birth periods were found to be protracted and separate with 14.5 months gestation. Lactation lasts at least 2 years; thus, the reproductive cycle encompasses 3 years.

These studies show that few corpora retained in the ovaries represent completed pregnancies, most resulting from either unfertilized multiple ovulations during annual estrus or from accessory corpora.

Underestimating age of female sexual maturity and overestimating reproductive rate have resulted in excessive productivity estimates for *D. leucas*.

Growth and Development

Age Determination

There has been considerable controversy regarding deposition rates of bone and dentine among Odontocetes (Sergeant 1959; Khuzin 1961; Klevezal and Kleinenberg 1967; Jonsgard 1969; Brodie 1969a). A comprehensive study of beluga bone and tooth growth rates is being prepared for publication by the author. There will be little discussion of bone and tooth growth rates in this paper; however, in this study, two dentine layers and one mandible layer are considered to be annual depositions (Brodie 1969a).

Calf Growth Rates

The reason for discrepancies in published estimates of beluga growth rate appears to be the scarcity of comprehensive data for younger animals. When several criteria are considered, differences between younger age classes become obvious, though individual criteria may overlap.

Body length, maximum girth, weight, teeth, body scars, and parasites were considered for the sample of Cumberland Sound calves. Mother–calf associations were also used as evidence. The calves have been divided into groups based on initial impressions in the field.

Two full-term fetuses are included here for continuity, being of mean length 165 cm (156 and 174 cm) and mean weight 79 kg (63.7 and 94 kg). No layers were present in the unerupted teeth.

Seventeen newborn animals were of mean length 159.6 cm (SD = 10.1), mean maximum girth 108.3 (SD = 11.4), and mean weight 78.3 kg (SD = 17.4), with no layers yet deposited in the unerupted teeth (see head profile in Fig. 2A). No parasites were visible in the stomach or middle ear sinuses and the epidermis was void of scratches or scars. At least 10 still retained several centimeters of umbilical epidermal sheath.

Three calves tentatively classed as yearlings were of mean length 216 cm (209–225 cm), mean maximum girth 148.3 cm, (143–156 cm), and mean weight 187.8 kg (163.8–232 kg). No teeth had yet erupted in any of the three. Two narrow layers were present in the teeth of two animals; those of the third were not collected. These and all older animals carried middle ear parasites (*Crassicauda* sp.), and all but fetuses and newborn exhibited fresh and healed body scratches (see Fig. 2B for head profile). These calves had doubtless been exposed to the environment for more than a few days.

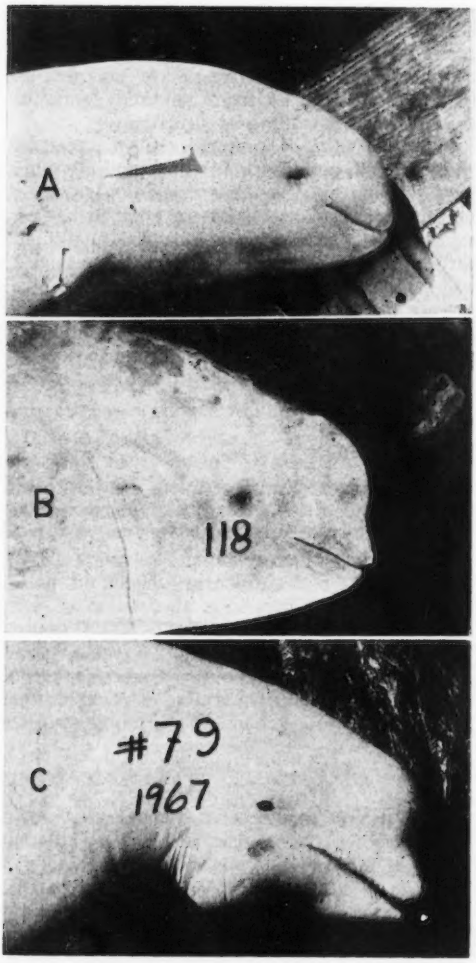

FIG. 2. A, Newborn beluga, slate grey in color, though underexposed here for contrast; B, yearling beluga, grey in color; C, adult female beluga, white in color.

From comparison of means of 17 newborn and three older calves, length increase was 56.4 cm (35.3%), maximum girth increase 40 cm (37%), and weight gain 109.5 kg (140%). These differences, coupled with the body scars and parasites, are evidence that these three animals were not of recent birth. The birth period for beluga in Cumberland Sound has been fixed as late July–early August, and these calves were therefore 12 months old; one was accompanied by a lactating animal whose most recent corpora indicated parturition the previous year.

The next group considered is composed of three animals with four narrow dentine layers present and mean body length of 250 cm (248–252 cm), maximum girth of two animals 167 and 168 cm, and weight of one animal 268 kg. The length and girth measurements being similar, the single weight is representative. If these animals are 2 years old, the annual gains compared to yearlings are 34 cm in length (15.8%), 19.2 cm in maximum girth (11.5%), and 79 kg (42.1%). In one animal, four teeth were beginning to pierce the gum, another had several piercing, and the third had no teeth erupting.

Comprehensive data are lacking on 13 calves larger than those judged to be 2 years old. The teeth of these were in the early stages of eruption, with between 2 and 3 mm of the tip penetrating the gum. Tooth layers of nine calves ranged between six and eight narrow layers. Mean body length was 277.9 cm (258–298 cm), and mean maximum girth of five calves was 179.6 cm (162–190 cm). Using the formula (Sergeant and Brodie, 1969),

$$\log \text{weight (kg)} = 2.605 \times \log \text{length (cm)} - 3.807$$

weights would range between 298 and 434 kg, suggesting that this group may be composed of more than one age class, probably 3 and 4 years old in view of the variation in number of tooth layers.

Apparently beluga in Cumberland Sound of lengths less than 300 cm include newborn and as many as four year-classes.

WEANING AND TOOTH ERUPTION

Tooth eruption begins late in the 2nd year, with one instance of eruption beginning after 3 years. Teeth may erupt first in either jaw with partial eruption in both jaws by the 3rd year (see also Dergerbøl and Nielsen 1930).

Milk is apparently the mainstay for the 1st year, supplemented with captured food during the

2nd. First evidence of predatory feeding was found in the stomach of a 209-cm yearling (teeth unexposed) containing one squid beak, pieces of worm case, and several 1–2-cm stones.

Physical Maturity

Figure 3 shows the total sample of animals of which age in tooth layers is known. Due to tooth wear, all tooth layer counts over 16 are underestimates, the discrepancy increasing with the increase in layers retained (Brodie 1969a). Though the curves are fitted by eye, sexual dimorphism is suggested (Kleinenberg et al. 1964; Sergeant and Brodie 1969).

Mean length of males with more than 20 tooth layers was 427 cm, and of females 362 cm. These lengths may approximate those of physical maturity attained after 10 years of age.

Whitening of female beluga in Cumberland Sound occurs after 6 years, and of males after 7 years. The white color can be used in the field to establish at least a minimum age and to indicate that the animal is near physically mature size (Sergeant and Brodie 1969). Based on tooth and mandibular layering (Brodie 1969a), maximum life span is 30 years.

Reproduction in Females

General Considerations

Beluga, like all Cetacea, have a bicornuate uterus and two functional ovaries. Multiple births are uncommon (Vladykov 1944; Kleinenberg et al. 1964). Presence of corpora lutea or corpora albicantia is considered a reliable criterion of puberty. The corpora albicantia of larger Cetacea are unusual in that they remain as distinguishable bodies in the ovary for the life of the animal. Dempsey and Wislocki (1941) suggest that this is a result of vascular supply limitations in a large corpus luteum.

Laws (1961) divided the regression of corpora lutea of fin whales (*Balaenoptera physalus*) into three stages: stage 1 corpora have undergone least regression and are apparent on the ovary surface; luteal tissue has begun to degenerate and connective tissue is conspicuous. Stage 2 corpora have a more obvious fibrous structure due to continued shrinkage. (In beluga, these are not as easily discernible on the ovary surface.) Stage 3 corpora are in the final stage of regression and connective tissue is the most obvious structure.

Since the time of capturing beluga coincided with the birth period, corpora lutea and albicantia were indistinguishable, the categories separated only by hours or days before or after birth. Considering this, the stages are expanded to include: early stage 1, late stage 1, early stage 2, late stage 2, and stage 3.

Since the age of calves can be used with some confidence in interpreting ovarian condition of their presumed mothers, it is important to note associations of beluga (usually from one to three animals) captured in passive nets.

Sexual Maturity

The female indicating earliest maturity was captured beside a 139-cm calf, had recently calved,

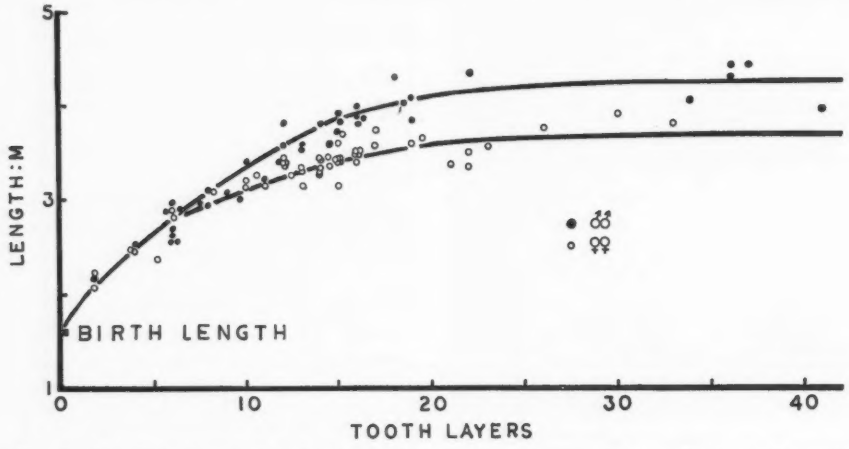

FIG. 3. Lengths in meters plotted against age in tooth layers present (not considering tooth wear). Mean birth length is indicated.

and had one 25-mm corpus albicans in early stage 1 and one 7-mm corpus in stage 2, the latter probably from an unsuccessful ovulation immediately before impregnation. The 14.5-month gestation period would place this animal's first estrous cycle at an age of eight tooth layers.

By excluding one atypical animal, immature at an age of 15 tooth layers, and back-calculating to account for gestation of nine animals with low corpora counts, the age of sexual maturity would range between 9 and 11 tooth layers, with a mean of 10. Thus, sexual maturity is attained, on the average, at 5 years and at 85% of the physically mature length.

Accessory Corpora Lutea

In *D. leucas*, accessory corpora result from luteinization of a second ruptured or unruptured follicle in addition to the corpus associated with the fertilized ovum. Neither degenerates during gestation (though see Nalbandov 1964 p. 261).

In Cumberland Sound during early August four females were captured with newborn enmeshed beside three. All adults were lactating, had distended cornua, and had twin corpora in early regression (three had additional older corpora). A gravid animal was captured several days earlier, with a 174-cm, 94-kg fetus. Two corpora lutea were present in the left ovary (47 and 29 mm in mean diameter).

Such complementary corpora in beluga have been used as evidence for postpartum breeding (Kleinenberg et al. 1964). This is ruled out for the gravid individual, and there was no evidence of pregnancy among the postpartum animals.

All corpora in question were probably formed during the previous breeding season 14–15 months prior to capture, as none had degenerated, and corpora lutea gravidans inhibit follicle growth during gestation. Of 39 females, either full-term or recently calved and with recent or functional corpora obvious by size and structure, five had accessory corpora gravidans.

Accumulation of Corpora Albicantia with Age

Total corpora lutea and corpora albicantia were plotted against age of each animal in tooth layers (Fig. 4). Due to tooth wear, all ages over 16 layers are underestimates (Brodie 1969a), the discrepancy increasing with age. The uncertain age of older animals discourages calculation of corpora accumulation rates by regression analysis. The variation in numbers of corpora in animals of similar ages has more meaning. Four groups of animals (Fig. 4) can account for the discrepancies in accumulation rates:

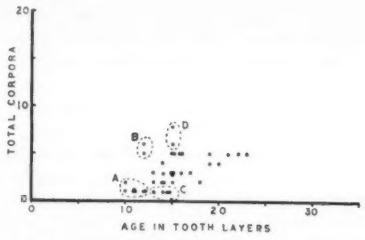

FIG. 4. Total number of corpora lutea and corpora albicantia plotted against the age of that individual in tooth layers present (tooth wear not considered). Shown encircled: A, young animals with few corpora; B, young animals with many corpora; C, older animals with few corpora; D, older animals with many corpora.

(A) young animals with few corpora, (B) young animals with several corpora, (C) older animals with few corpora, and (D) older animals with several corpora.

Of seven animals in group A, two were in the early stages of first pregnancy, with one corpus luteum (42 mm diam with 204-mm fetus; 42.3 mm with 203-mm fetus). Four others had recently calved and were lactating; three of these had single corpora albicantia in early stage 1 (21, 28, 37.7 mm diam), and the remaining animal had two corpora albicantia, 25 mm in early stage 1 and 7 mm in early stage 2. The latter probably resulted from an ovulation preceding impregnation on the second ovulation of a cycle, and could not have represented a prior cycle as the animal was too young. The seventh animal had one corpus, but data were insufficient to allow speculation as to its origin. Thus, group A is composed of animals impregnated on first ovulation and of one impregnated on second ovulation of its first estrous cycle. Formation of additional corpora was inhibited during gestation and the greater part of lactation resulting in low corpora numbers.

Group B was composed of two animals with 12 tooth layers. One female had five corpora, had just calved, and had accessory corpora in early stage 1, one stage 2, and two stage 3; she was too young to have completed a reproductive cycle in addition to this gestation period. The other had six corpora, was lactating and, judging from recent corpora (three in stage 2), had calved 1 year prior to capture. She also was too young to have completed a cycle previous to the recent pregnancy. Summarizing, both females in group B were too young to have completed more than one reproductive cycle, and high corpora numbers are attributed to unfertilized ovulations and accessory corpora gravidans.

Group C was composed of five animals with 13–15 tooth layers and one or no corpus present. Two females had recently calved, were lactating, and had an early stage 1 corpus; sexual maturity was retarded somewhat, though both were impregnated on first ovulation. Two other lactating animals had an early stage 2 corpus; they had calved the previous year, and therefore became sexually mature 26.5 months prior to capture (12 months lactation plus 14.5 months gestation). One sexually immature animal was included. Thus, group C can be accounted for by late maturity with recent parturition or by sexual maturity at the usual age with parturition a year prior to capture.

Group D contained two animals with 15 tooth layers and six to eight corpora, both having recently calved. One had two stage 1 corpora (accessory corpora), three stage 2, and three stage 3 corpora. The other had one stage 1 corpus, three stage 2, and two stage 3 corpora. Although both could have completed reproductive cycles prior to the latest pregnancy, it is equally likely that they were not impregnated during the first cycle and conceived the following year. Summarizing group D, many unfertilized ovulations and at least one accessory corpus are indicated.

The point to be stressed is that a minority of the corpora albicantia present represent pregnancies and are poor indicators of production.

Symmetry of Ovary Function

In this test, only data from females in which the side of location of the ovary ovary was known were used. Corpora in all stages in 43 animals totalled 99 in the left ovary and 73 in the right. A chi-square test using 1 degree of freedom showed nonsignificant differences at the 1% level ($0.05 > P > 0.02$). The same animals were then grouped randomly until total corpora on either side exceeded 10. Six groups were formed and their chi-squares for parity summed, yielding a significant difference ($0.01 > P > 0.001$). These tests indicated variability between groups resulting in nonsignificant differences when totalled, meaning that either ovary may be dominant with respect to corpora number.

Ohsumi (1964) concluded that, in beluga, 70% of the corpora accumulate in the left ovary and the left matured earlier. This was based on 16 animals from Hudson Bay.

To test for differences in time of follicle maturity between left and right, data were used from females with low corpora counts and in which the most regressed corpus was obvious. Of the 29 females in this category, earliest follicle maturation occurred in the left ovary of 19 and in the right of 10. A chi-square test with 1 degree of freedom showed a nonsignificant difference from parity ($0.10 > P > 0.05$).

To test for symmetry of fertilized ovulations, all animals with corpora lutea gravidans or corpora albicantia gravidans from recent parturitions were used. Of 36 females, 27 had the corpus in the left. A chi-square test using 1 degree of freedom showed a significant difference from parity ($0.01 > P > 0.001$).

The only significant finding from the Cumberland Sound animals is the indication that the products of the left ovary are either more viable or are more frequently fertilized.

Gestation

A straight-line method was used to calculate the gestation period (Huggett and Widdas 1951; modified by Laws 1959) (Fig. 5). Fetal lengths, when sampled during the early and midsummer months from any beluga population, form two size groups. Lengths of newborn, most retaining several centimeters of umbilical epidermal sheath, were included with the fetuses. Full-term fetuses and newborn were plotted twice, i.e., 12 months apart to continue the curve in proper perspective (see also Sergeant 1962b and Best 1968).

The time intercept is approximately 400 days prior to the known birth period of late July–early August. As the straight line does not take into consideration early growth, the originators of this method adjust for this by multiplying by 1.10,

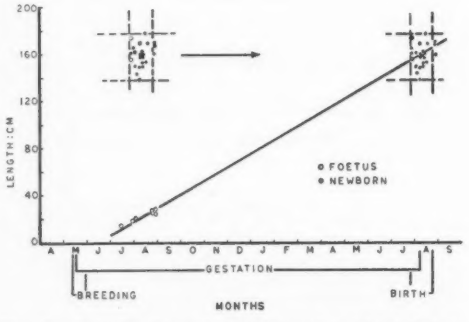

FIG. 5. Lengths (in cm) of fetal and newborn beluga plotted against time of capture. Full-term fetal and newborn lengths have been plotted twice, 12 months apart to give a continuous curve. Range of birth lengths and approximate calving period are indicated by the broken lines. Early fetal growth is not included in the straight-line fit.

which gives an estimated gestation of 14.5 months for beluga. This would place the peak of breeding in mid-May. Laws (1959) and Sergeant (1962a) calculated 13–14 months gestation for beluga from Hudson Bay. All estimates exceed the 11.5–12 months proposed by Kleinenberg et al. (1964).

The small fetuses found during the calving period (Fig. 5) are at least several weeks old and must have been conceived before ice conditions allowed beluga to enter Clearwater Fiord area. The smallest fetus discovered was 155 mm in length, on July 13. Kleinenberg et al. (1964 p. 260) state "Increase in (fetal) length is 5 cm in the first month, more than 7 cm in the second and more than 12 cm in the third month." On the basis of these estimates, conception would have occurred in early May, so that gestation is of the order of 14.5 months. This is further evidence against annual production.

Postpartum Ovulation and Conception

Of the Cumberland Sound sample, five animals had follicles enlarged to a noticeable degree (9.3–19.3 mm, mean 12.1 mm). Two had recently calved, two had calved 1–2 years earlier, and all were lactating. The fifth animal had not calved for at least 2 years, was not lactating, and had one 12.0-mm follicle.

Had postpartum breeding been common, there would have been a wide variation in early fetal size, as a result of the known breeding in May and the supposed breeding during the calving period (Kleinenberg et al. 1964), but such variation was not observed.

Lactation

Lactating beluga were captured in nets accompanied by calves in the 209–225 cm range, judged to be yearlings. Three lactating animals (none pregnant), with regressed corpora albicantia, were captured near calves judged to be 2 years old. Three lactating animals in the early stages of pregnancy were captured carrying 155–209-mm fetuses, indicating lactation in this state is not inhibited; they had regressed corpora albicantia and were old enough to be multiparous. The possibility of lactation stimulated by early pregnancy is ruled out, since only animals capable of prior pregnancy were lactating. One young female with a 203-mm fetus was not lactating and as evidenced by the presence of a single corpus, was primiparous.

As lactation lasts at least 2 years and is not inhibited by at least the early stages of the following pregnancy, this increases my previous estimate (Brodie 1969b). Dergerbøl and Nielsen (1930) suggested lactation exceeding 1 year, Vladykov (1944) approximately 6 months, and Kleinenberg et al. (1964) a maximum of 5 months.

Reproductive Cycle

The reproductive cycle is defined here as the mean period between onset of consecutive pregnancies. The gestation period has been shown to last approximately 14.5 months. Calves are nursed in excess of 24 months; however, the mother may be pregnant during the later stages. Since breeding is in May, a 36-month cycle is indicated (Fig. 6).

Reproduction in Males

Calculated testes volumes are plotted against age of the animal in tooth layers (Fig. 7). An abrupt increase in testes volume is apparent at about 90% of the probable age of physical maturity. The rapid testes growth rate is also demonstrated by the paucity of intermediate volumes (see also Sergeant 1962b).

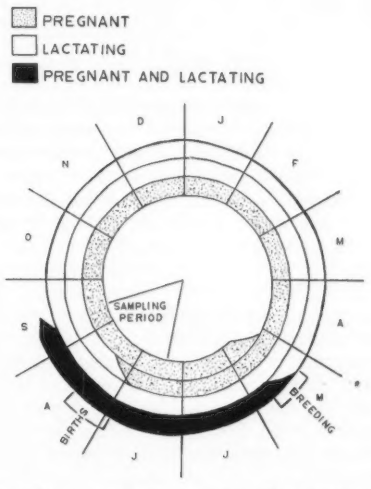

FIG. 6. The reproductive cycle of *Delphinapterous leucas* reconstructed from the restricted sampling period. Time in months is indicated. Moving clockwise and outward: (1) females in the early stages of their first pregnancy and not lactating; (2) females either full-term and beginning to produce milk or recently calved and lactating; (3) females with no recent corpora, lactating and accompanied by calves judged to be yearlings; (4) females in early pregnancy and still nursing calves judged to be 2 years old. The 36-month reproductive cycle is included here, being the time between onset of consecutive pregnancies. Breeding and birth periods are indicated.

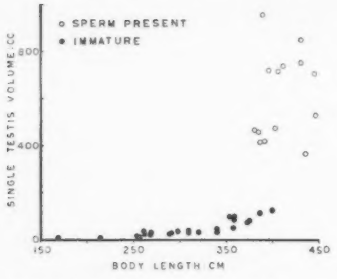

FIG. 7. Calculated volumes of a single testis plotted against total body length of that individual. Small amounts of sperm were present only in those testes larger than 360 cc volume.

Spermatozoa were not present in testes less than 130 cc volume, but small numbers were present in all testes that exceeded 360 cc volume. The youngest animal with increased testis volume and spermatogenic activity had 14 layers in its slightly worn teeth, indicating sexual maturity after 7 years. All animals older than this were mature; thus, sexual maturity is attained by 8 years of age in *D. leucas*.

Behaviour

Based on personal observation and that of Eskimo hunters, all or almost all Cumberland Sound beluga inhabit Clearwater Fiord during late July to mid-September. All age classes of both sexes are represented, though there is some segregation of adults (however, see Kumlien 1879). Full-term and postpartum females separate from the main herd and move along close to shore or into smaller, sheltered bays. The reasons for occupancy of the fiord may be thermal efficiency (Sergeant and Brodie 1969) and escape from predation. The apparent segregation of sexes within the fiord at this time suggests that this is not a period of breeding activity, and the absence of bastocysts or embryos resulting from possible July–August matings is evidence that there is no reproductive activity.

Postpartum females were accompanied by a new calf plus one, and less commonly two, nonwhite subadults, which could represent previous offspring. Maintaining kinship could be advantageous when feeding (Brodie 1969b).

Population Studies

Population Size, Past and Present

On August 30, 1967, an aerial census of Clearwater Fiord beluga indicated 476 and 455 animals on two consecutive counts.

Observations on diving behaviour were made from cliffs. An average sequence consisted of 5.5 shallow dives, during which time the animals were constantly visible, followed by a single dive of greater duration and depth, when the animals were not visible. The mean duration of the sequence was 122 sec, during 87 of which the whale was visible.

Animals at depths beyond visibility are calculated relative to visible numbers as 35/87 or 0.40, which must be added to the visible part of the population; thus, $465 + (465 \times 0.40) = 652$.

Due to the small size and dark coloration of the newborn and yearlings, it is assumed that the majority of these were not observed. Using 10%[2] as the number of newborns present and (considering 1st-year motality) 8% as the number of yearlings present, the approximate population in late August 1967 would be $652 + (652 \times 0.18) = 769$.

The aerial census figures suggest that the population must have been greatly reduced in recent years, because until the early 1960's, this population was exploited commercially. Sergeant (1962a) estimated the annual catches in Cumberland Sound from the recorded oil yield (using the Churchill, Man., plant yield of 30 gal per beluga). If the larger size of Cumberland Sound beluga (Sergeant and Brodie 1969) would make 40 gal a closer estimate, the estimated mean annual catch between 1948 and 1960 is reduced from 163 to 134 animals. The author estimates 40 additional animals are taken noncommercially by local Eskimo hunters annually.

A total overall herd production of 8% per year can be used as a basis to calculate earlier population size from the 1967 census. Calculating back to 1948 (the first year of recorded oil yield) prior to the summer's catch, the population must have been about 1750 animals. The estimated annual kill of 174 animals (10%) from commercial and noncommercial hunting was apparently greater than the annual herd production between 1948 and 1960. Presumably, the herd remained intact, returning to Clearwater Fiord each year.

There are many assumptions in the above calculations, justified in part by Soper's (1928) report of commercial hunting in Clearwater Fiord. In 1923, about 600 beluga were killed by man-forced stranding, and about 800 in early July 1924. On July 29, 1924, "large numbers" of the remaining beluga

[2]Based on: 50% of herd females; females mature at a mean of 5 years, with adult mortality unknown; 3-year reproductive cycle; thus, less than 33% of mature females are producing annually (considering age of puberty plus adult mortality) (see also Sergeant (1962a).

were observed in Clearwater Fiord, and in the summer of 1925 "large numbers" were killed. Considering Soper's kill estimates (older Eskimos recall these mass slaughters), the population must have been large to have 600 and 800 taken in two summers, plus a large kill a year later. Presumably, there was a noncommercial kill by the many active Eskimo camps of that period.

The conclusion is that the initially large beluga population of the early 1920's was rapidly reduced, remnants of this herd being less rapidly reduced by the smaller commercial fishery of the 1940's and 1950's. By the 1960's, noncommercial hunting and natural mortality had depleted much of the herd production, indicating that production is not high.

Productivity

A simple comparison of potential rate of increase can be made if no mortality or density dependence is assumed and if multiple births are rare, by solution of the equation for instantaneous rate of increase (r):

$$1 = \sum_{t=\alpha}^{\omega} b_t \, e^{-rt},$$

where α is the age of first birth (6 years in this study, 4 years in Kleinenberg et al. 1964), b_t is number of female young at age t (0.5 every 3 years in this study, and 0.5 annually except every 5th year in Kleinenberg et al. 1964), ω is the age of last birth, arbitrarily assumed to be 21 (see Kleinenberg et al. 1964 p. 279), and the estimates of r are 0.09 and 0.21. Production is therefore only 43% that of Kleinenberg et al. (1964).

This difference deserves resolution if intensive exploitation is planned in future.

Discussion

Qualitative analysis of a small sample of *Delphinapterus leucas* has helped to resolve aspects of population size in which variability and misinterpretation can lead to gross errors in generalizations. Body-size differences between populations (Sergeant and Brodie 1969), and variation in climate or ice conditions throughout the Arctic contribute to the problem.

Most of the findings have been compared with those of Kleinenberg et al. (1964), the most comprehensive published on the species. Age determination has been the most controversial aspect in beluga studies (Sergeant 1959; Khuzin 1961; Klevezal and Kleinenberg 1967 (review); Jonsgard 1969 (review); Brodie 1969a). The grouping of one or more year-classes with newborn has the obvious result of leading to underestimated ages of adults and of implying prodigious growth rates of calves. Examination of data from Doan and Douglas (1953) suggests they had grouped newborn and yearlings. Kleinenberg et al. (1964) classed all calves up to 280 cm as being less than 5 months old, though this study shows that such a size range includes newborn and 3-year classes. The phenomenal weight gain implied (as high as 500%) by Kleinenberg et al. would place considerable demands on a nursing mother over a 5-month period (herself weighing between 650 and 1150 kg).

Tomilin (1957) quotes length gains between year-classes as 62–110 cm during the 1st year, 22–102 cm during the 2nd, and 23–103.5 cm during the 3rd and subsequent years. These broad ranges of length gains would each include two and even three year-classes.

The lifespan of beluga has been calculated at 32 years (Sergeant 1959), 40 years (Khuzin 1961), and 20–32 years (Kleinenberg et al. 1964). Studies of tooth and mandible layers of beluga suggest 30 years as maximum (Brodie 1969a).

Kleinenberg et al. (1964) considered all corpora albicantia as representing completed pregnancies, thus implying a high reproductive rate. This study shows that most result from unfertilized multiple ovulations during single annual estrus cycles, plus the occurrence of accessory corpora lutea gravidans. Production could be negatively correlated with number of corpora albicantia, i.e., an older animal with few corpora could have been more productive than one with many corpora, because many of the former's estrous cycles would be inhibited by gestation and lactation. The numbers of corpora present in this species cannot be used with any confidence in age determination. There may be some stability of the reproductive cycle among older females, such as better coordination of ovulations with the mating activity, or a selection of older females by the breeding males. Kleinenberg et al. (1964 p. 12) state that females with one to four scars on the ovaries, (pregnant up to four times) are most numerous, and that the sharp decrease in females with five or more scars apparently indicates elimination of the older age-groups.

When found, accessory corpora albicantia in early stage 1 were considered by Kleinenberg et al. (1964) as representing a corpus albicans of a recent birth plus a new corpus luteum associated with (the supposed) recent conception. This study showed that both corpora originated from one pregnancy and that there was no evidence of either postpartum breeding activity or conception.

The presence of accessory corpora had been interpreted by the above authors as evidence for annual calving. To account for the nonpregnant, lactating animals they proposed a 4–5 year series of annual pregnancies gradually going out of phase with the supposed postpartum breeding season (because of the 2–4-week lag in conception after birth). A 1-year resting period thus follows failure to conceive. In the present study, animals in this state were found to be nursing 1- and 2-year-old calves.

Though the sample of fetuses from Cumberland Sound is small, it can be suggested that the degree of scatter of fetal lengths (fetuses of the same breeding period) at a given time may reflect the severity of regulation by the local climate. Selection would be for animals that conformed best to the ideal calving period. The degree of scatter is greater (though from larger samples) among temperate whale populations described by Sergeant (1962b) and Best (1968).

Acknowledgments

I thank Dr D. E. Sergeant for his valuable advice and assistance throughout the program, and Dr A. W. Mansfield for checking the manuscript. Dr I. A. McLaren advised me while at Dalhousie University. In the field, assistance was given by staff of the Department of Indian Affairs and Northern Development and of the Hudson's Bay Company. I am indebted to many hunters from Pangnirtung who assisted me throughout the fieldwork.

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NOTES

Perception of Polarized Light by Yearling Sockeye Salmon (*Oncorhynchus nerka*)[1]

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DILL, P. A. 1971. Perception of polarized light by yearling sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 28: 1319–1322.

Food-conditioning experiments conducted in the laboratory demonstrated that groups of yearling sockeye salmon (*Oncorhynchus nerka*) can discriminate between vertical and horizontal planes of linearly polarized light. Removal of the adipose eyelid, a possible analyser of polarized light, did not abolish the response. The findings support the hypothesis that these fish can use polarization patterns for orientation during their seaward migration.

DILL, P. A. 1971. Perception of polarized light by yearling sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 28: 1319–1322.

Des expériences de conditionnement de nourriture poursuivies en laboratoire ont démontré que des groupes de saumons sockeye (*Oncorhynchus nerka*) d'un an sont capables de distinguer entre un plan vertical et un plan horizontal de lumière polarisée linéairement. L'ablation de la paupière adipeuse, analyseur possible de lumière polarisée, n'a pas supprimé la réaction. Ces résultats corroborent l'hypothèse que ces saumons utilisent les modalités de la polarisation pour s'orienter au cours de leur migration vers la mer.

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FIELD observations at Babine Lake have shown that yearling sockeye salmon (*Oncorhynchus nerka*) migrating seaward from their nursery areas move along the most direct route to the exit of the lake (Johnson and Groot 1963; Groot 1965) indicating that young salmon are able to orient in time and space over extended periods.

Investigations by Hasler et al. (1958), Braemer (1960), and Schwassmann (1960) suggest that orientation during long-term migrations could depend on the use of the sun as directional reference. Groot (1965) found evidence to support this hypothesis in migrating yearling sockeye salmon. Sockeye salmon yearlings, however, migrate primarily during twilight periods (mostly at dusk) indicating that other cues besides the sun must be of importance. Some of Groot's experimental results suggest that the polarization pattern of the sky, a predominant feature of twilight, may be used in orientation.

This report gives results of food conditioning experiments designed to determine whether yearling sockeye salmon can discriminate between different planes of polarized light.

Materials and general methods — The sockeye salmon used were caught on April 28, 1964, at the outlet of Great Central Lake on Vancouver Island, British Columbia, during their seaward migration. They were transported to the Fisheries Research Board of Canada Nanaimo Biological Station and held in an outdoor tank until tested. Experimental fish were maintained on a light–dark cycle (14L:10D) by a 40-w incandescent source which was mounted above the center of the conditioning tank and which cast more than 100 ft-c on the water surface (Photometer 200, Photovolt Corp., New York).

The octagonal conditioning tank (Fig. 1A) (1 m diameter and 15 cm high) was constructed from clear acrylic plastic 1 cm thick. Filtered fresh water, with no

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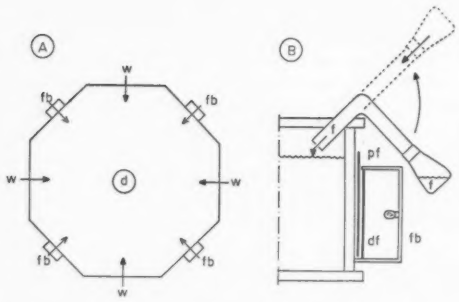

FIG. 1. (A) Plan view of conditioning tank. Arrows indicate water inflow (*w*) and food entry above filter box (*fb*). Water exits by drain (*d*). (B) Side view of one filter box containing diffusing filter (*df*) and polaroid filter (*pf*). Food entry (*f*) is also indicated.

particles larger than 5 μ, was introduced by four inlet hoses and removed through a central drain. The water level was maintained at 12 cm. The temperature fluctuated with the water supply between 10 and 14 C.

Four feeding tubes were located on four sides of the octagonal tank (Fig. 1A). Each consisted of a piece of rubber tubing (15 cm long, 1.5 cm outside diameter) mounted at a 45° angle to the water surface (Fig. 1B). Prior to a trial the tubes were bent so that a 10-ml flask could be attached to the end of the tube without spilling its contents. When the feeding tubes were released by the observer, the bent rubber straightened and contents of the flasks poured into the conditioning tank.

A box containing a linear polarizer (9.5 × 9.5 cm of HN 22; Polaroid Corporation, Cambridge, Mass.) was mounted below each feeding tube. Unpolarized light from an incandescent source (Northern Electric 51) inside each box passed through a diffusing filter of sanded acrylic plastic and through the linear polarizer, to emit less than 0.5 ft-c into the conditioning tank (Fig. 1B) (less than 1/100 of the light emitted from the light–dark cycle source). The fish were observed from beneath the tank through one-way glass.

A conditioning experiment began by introducing eight fish (10–15 cm long) into the tank. The fish were then deprived of food for 3 days before conditioning began. Training was divided into two distinct phases. In the first phase fish were trained to go to the area of the filter boxes when filters were illuminated. To accomplish this, food was introduced at the filter area 3 sec after the lights were turned on. After all eight fish went to a filter area during the 3-sec interval in anticipation of food, the second phase of training began.

For the second phase of training polaroid filters were placed in the filter boxes. The plane of polarization was vertical for one filter and horizontal for the other three. The filter box containing the vertical plane, and the filter used for this, were chosen at random for each trial. During each trial the four filter lights were illuminated, there was a 3-sec interval during which the number of fish arriving at each filter area was counted, and then food was introduced at the vertical filter area only.

Two experiments were performed using the above method, the first on eight intact fish and the second on eight fish with their adipose eyelids removed. This tissue normally covers two thirds of the circumference of the eye with anterior and posterior portions extending over the cornea (Walls 1942) and, as it has strong birefringent properties (Stewart 1962), it may be an analyser of polarized light. Not more than five trials were conducted each day for the duration of each experiment.

Experiment 1 — Eight fish were placed in the apparatus on June 26. After about 15 trials, all fish went consistently to the filter areas when the lights were turned on. The first phase of training was then continued for an additional 25 trials. The polaroid filters were then introduced into the experiment and the second phase of training was conducted for 11 days.

Rate of learning was estimated by plotting cumulative numbers of responses of the eight fish to the vertical and each of the horizontal filters during 54 trials (Fig. 2A). The average response of 2.0 to each filter up to trial 29 indicated that fish were responding at random (two of the eight fish to each of the four filters). Beyond this point the average response to vertical and horizontal filters diverged. The average number of responses in each trial to the vertical filter from trial 29 to the end of the experiment rose to 3.4, while that to the horizontal ones dropped to 1.5. Thus juvenile sockeye salmon are capable of discriminating between filter boxes containing vertical and horizontal polarizers.

Experiment 2 — Eight fish without adipose eyelids were introduced into the conditioning tank on July 20. The method of training was the same as that for the first experiment except that the first phase was conducted for 15 trials.

The cumulative numbers of times that the eight fish responded to the vertical and each of the three horizontal filters during 36 trials (Fig. 2B) were similar to those of the first experiment. Until trial 15 the average number of responses to each plane of polarization was 1.8. The average response to the vertical filter from trial 15 to trial 36 rose to 2.8, while that to the horizontal planes dropped to 1.5 per trial. Thus, the ability of fish to discriminate between filter boxes with vertical and horizontal planes of polarization was not affected by removal of their adipose eyelids.

In order to ensure that fish were responding to the plane of polarization and not to undetected cues such as marks on filters, odours, or sounds, the second experiment was continued with the

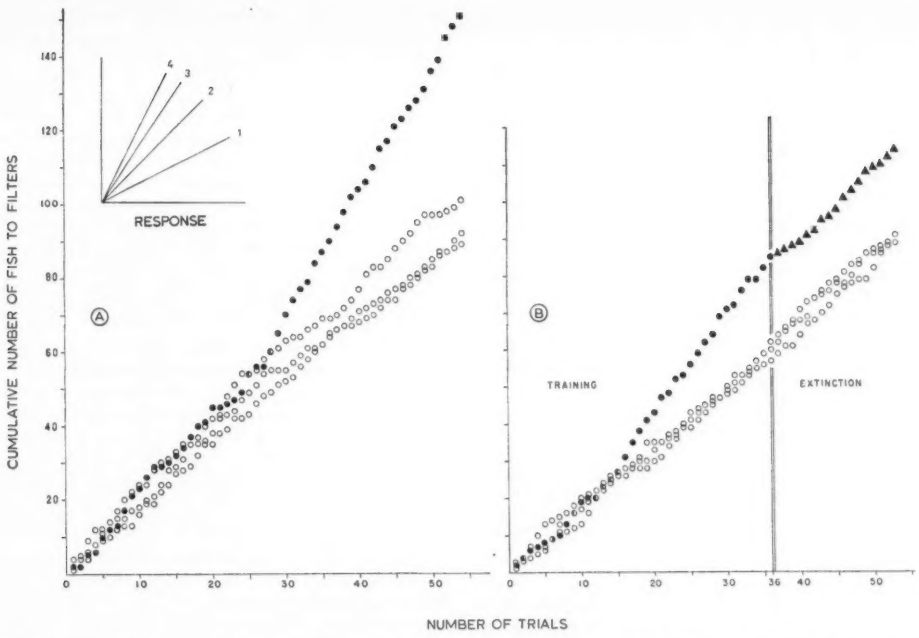

FIG. 2. Cumulative numbers of times that the eight fish responded to the vertical filter (solid symbols) and each of the three horizontal filters (open symbols) during (A) experiment one and (B) experiment two, and to the area of the vertical filter rotated to a horizontal position (triangles) during the last part of experiment two (circles half solid and half open indicate coincident points). The slopes of the cumulative data with a given number of responses to a filter are shown in top left of A.

vertical filter rotated to a horizontal position, i.e. all filters were then horizontal.

Further trials resulted in a reduction in number of responses to the area that received food (from an average of 2.8 to 1.70 per trial) and an increase in the average number of responses to the horizontal filter area (from 1.60 to 1.74 per trial) (Fig. 2B). Thus, the average response per trial after the vertical filter was rotated dropped to a level similar to that expected at random.

Discussion — Results of the conditioning experiments presented here indicate that sockeye salmon are capable of discriminating between vertical and horizontal planes of polarized light. Among vertebrates, only a freshwater teleost of the genus *Pterophyllum* (Waterman 1959) and saltwater fish *Zenarchopterus dispar* and *Z. buffoni* (Waterman and Forward 1970) have been reported to show indications of orientation to polarized light. Homing pigeons (Montgomery and Heinemann 1952) and Pacific herring, *Clupea pallasi* (K. W. Stewart, personal communication), have shown no evidence of discriminating between patterns of polarized light.

The mechanism of discrimination could have been extra-ocular if the fish could detect differential reflection, refraction, or scattering of polarized light in the environment. This possibility is unlikely, firstly because filtering the water removed suspended particles that normally cause the greatest amount of light scattering (Jerlov 1963). Secondly, by conducting experiments under high general illumination compared to the low illumination emitted at the polarizers, differential reflection and refraction of polarized light were obscured.

Alternatively, discrimination of the plane of polarization must be intra-ocular, i.e. light is absorbed differentially for different angles of the plane of polarization. Although the adipose eyelid has the potential to be an analyser, results of experiment two demonstrate that it was not involved in discrimination of horizontal and vertical polaroids. Visual pigments themselves may be capable of absorbing differentially for different directions of the plane of polarization. This type of dichroic

analyser was found to be responsible for analysis of polarized light by octupus (Moody 1964). Waterman et al. (1969) have made direct measurements of receptor dichroism in the crayfish eye. Such a mechanism, however, has yet to be demonstrated in the vertebrate retina.

An environmental characteristic, such as the plane of polarization, can be a directional aid during migration only if the animal is able to orient its body axis in relation to the cue. No conclusive evidence has been documented on the role of polarized light in orientation of vertebrates. Results presented in this paper support the hypothesis (Groot 1965) that yearling sockeye salmon may use the plane of polarization of the blue sky for maintenance of direction during their seaward migration in lakes. (Groot 1965).

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A Quantitative Sampler for Aquatic Phytomacrofauna

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MACKIE, G. L., AND S. U. QADRI. 1971. A quantitative sampler for aquatic phytomacrofauna. J. Fish. Res. Bd. Canada 28: 1322–1324.

A sampler was designed to quantitatively sample the invertebrates associated with submerged aquatic macrophytes. The advantage of this design over the previous ones is that it excludes benthic macrofauna.

MACKIE, G. L., AND S. U. QADRI. 1971. A quantitative sampler for aquatic phytomacrofauna. J. Fish. Res. Bd. Canada 28: 1322–1324.

L'article décrit un appareil destiné à l'échantillonnage quantitatif des invertébrés associés aux macrophytes aquatiques submergés. Contrairement aux modèles antérieurs, le nouvel échantillonneur a l'avantage d'exclure la macrofaune benthique.

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A quantitative investigation of the animals on submerged aquatic macrophytes (phytomacrofauna; Gerking 1957) was undertaken during a limnological survey of the Ottawa River. Most of the methods previously used for determining the numbers of invertebrates on aquatic plants (Rickett 1922; Rawson 1930; Krecker 1939; Deevey 1941; Andrews and Hasler 1943; Ball 1948) have quantitative inadequacies. The most recent attempt at developing a quantitative sampler for aquatic macrophytes and their associated fauna was that by Gillespie and Brown (1966). However, as we interpret the operation of their sampler while it is resting on the substrate, the sampler takes not only the plants but a large portion of the substrate as well. Consequently, it would be impossible to distinguish between the benthic fauna and the phytomacrofauna. Gerking (1957) attempted to separate the two by enclosing a small area of the substrate, with its plants, in a rectangular box. There are certain limitations in his technique. He hand-picked the plant specimens and, in doing so, may have shaken some animals from the plant. These animals could then be incorrectly labelled as benthos in subsequent substrate samples. Moreoever, the Gerking sampler was limited to depths less than 1 m. The requirements of the present study stipulated that the phytomacrofauna be taken independently of the benthic macrofauna and in depths greater than 1 m. To meet this need a new sampler was designed.

The sampler (Fig. 1*a* and *b*) consists of a cutter-blade box (A) with inside dimensions of 38 × 41 × 15 cm and a detachable tapered net (B) with outside dimensions of 38 × 41 × 60 cm and mesh of 0.37 mm. Heavy gauge aluminum is used for the frame (*c*) and blades (D1, D2, D3) of the sampler. A cutter-blade (D1) with a sharp edge is attached to the top of a support blade (D2) that contains a tripping bar (E). The tripping bar is pulled back through a hole (E1) in one end of the box and held in position by fitting the tripping mechanism (F) into a hole (F1) in the tripping bar. Two pieces of surgical rubber tubing are cut and made into two bands (G) of sufficient length to keep the blades tightly closed.

To load the sampler, the bands are streched into the slots (H) of the support blade (D2) and the impact blade (D3). For maximum safety, a pin (I) is inserted into a hole (J) in the tripping bar before applying the rubber bands. The net (B) is then inserted into the groove (K) on the cutter-blade box. After the safety pin (I) has been removed, a handle (P), hinged and locked at the tapered end of the net, is used to lower the sampler into a bed of aquatic vegetation.

When the sampler is in position, a messenger is sent down a guide wire (M) that is attached to the tripping mechanism. The impact of the messenger forces the tripping mechanism down to release the cutter blade,

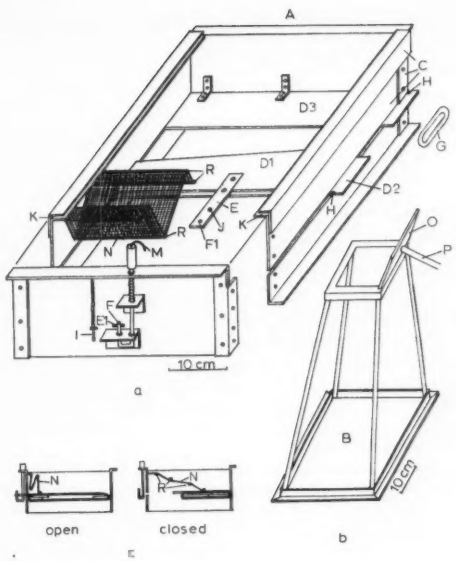

FIG. 1. (*a*) Construction of cutter-blade box; screen (N) incomplete; (*b*) frame of tapered net (net not shown); (*c*) closure of screen (N) in the cutter-blade box.

which then shears the plants as it approaches the impact blade. A screen (N) is hinged (R) to the cutter blade (Fig. 1*a* and *c*) and moves across with the blade to close the opening.

The sampler is then picked up, the rubber bands are removed, the blades are pulled apart, and the contents are washed into the tapered end of the net. After the net is detached from the box, the contents of the net are washed through the hinged door (O) into a container.

With sufficient length of guide wire and detachable lengths of handle, the sampler was easily used in 2-m depths. It was not tested in depths greater than 2 m because plants were absent beyond this depth in most parts of the Ottawa River.

The sampler worked extremely well in beds of *Myriophyllum* spp., *Elodea canadensis*, and *Vallisneria americana*. There was sufficient force behind the blades to shear a stick approximately 1 cm in diameter.

To test the efficiency of the sampler, a small area (10 m²) in the mouth of the Gatineau River near Gatineau Pointe, Que., was chosen. Duplicate samples of *Myriophyllum* sp., *E. canadensis*, and *V. americana* were taken with both a 15 × 15 cm Ekman grab (with a screen on top) and the present phytomacrofauna sampler. The animals were washed from the plants with a jet of tap water. Total volumes

TABLE 1. Average numbers of animals taken in two samples of three submerged macrophytes with the phytomacrofauna sampler and an Ekman grab. Analyses are based on 100 cc of each aquatic plant.

	Phytomacrofauna sampler			Ekman grab		
	Myriophyllum sp.	*Elodea canadensis*	*Vallisneria americana*	*Myriophyllum* sp.	*E. canadensis*	*V. americana*
Dugesia tigrina	84	45	23	84	69	9
Stylaria fossularis	36	39	12	9	6	3
Stylaria lacustris	9	12	9	3	3	11
Asellus sp.	0	0	0	0	3	0
Hyalella azteca	1593	1029	131	867	483	34
Gammarus fasciatus	9	24	5	3	6	0
Crangonyx pseudogracilis	0	0	3	0	0	0
Hydracarina	3	8	3	3	3	0
Ephemeroptera	0	0	3	0	0	0
Odonata	78	57	11	14	8	3
Lepidoptera	0	0	0	0	0	6
Tricoptera	24	6	3	6	0	0
Cricotopus sp.	15	0	3	9	0	0
Gyraulus parvus	18	30	14	15	9	3
Gyraulus hirsutus	0	3	0	0	0	0
Gyraulus deflectus	15	6	7	6	6	3
Promenetus exacuous	15	9	3	12	12	0
Physa gyrina	9	39	3	18	18	0
Amnicola limosa	36	78	19	21	21	12
Ferrissia parallela	0	0	9	0	0	12
Total:	1944	1385	261	1070	665	96

of plants were determined by water displacement; excess water on the plants was blotted dry prior to plant volume measurements. The number of animals per 100 cc of plant was then determined for each sampler (Table 1). The phytomacrofauna sampler evidently took more animals than did the Ekman grab for each species of plant tested.

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Postmortem Changes in the Glycogen Phosphorylase Activity of the Muscle of White Sucker (*Catostomus commersoni*) and Northern Pike (*Esox lucius*)

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MANOHAR, S. V., AND H. BOESE. 1971. Postmortem changes in the glycogen phosphorylase activity of the muscle of white sucker (*Catostomus commersoni*) and northern pike (*Esox lucius*). J. Fish. Res. Bd. Canada 28: 1325–1326.

Glycogen phosphorylase "*a*" activity in the muscle of northern pike (*Esox lucius*) decreased during 7 days of storage at 0 C. Under similar conditions, activity of this enzyme increased in the muscle of white sucker (*Catostomus commersoni*). The total amount of phosphorylase "*a*" + phosphorylase "*b*" remained constant during storage for 7 days in both species. The reduced phosphorylase "*a*" activity in pike muscle may explain the slow degradation of glycogen in the muscle of this fish during storage as compared to that in white sucker.

MANOHAR, S. V., AND H. BOESE. 1971. Postmortem changes in the glycogen phosphorylase activity of the muscle of white sucker (*Catostomus commersoni*) and northern pike (*Esox lucius*). J. Fish. Res. Bd. Canada 28: 1325–1326.

L'activité de la glycogène phosphorylase "*a*" dans le muscle du grand brochet (*Esox lucius*) diminue au cours d'un entreposage de sept jours à 0 C. Dans des conditions identiques, l'activité de cette enzyme s'accroit dans le muscle du catostome noir commun (*Catostomus commersoni*). Au cours d'un entreposage de sept jours, la quantité totale de phosphorylase "*a*" + phosphorylase "*b*" demeure constante chez les deux espèces. L'activité réduite de la phosphorylase "*a*" dans le muscle du brochet explique peut-être le lent catabolisme du glycogène dans le muscle de ce poisson, par rapport à celui du catostome noir, au cours de l'entreposage.

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THE activity of the enzyme glycogen phosphorylase in northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) was studied in an attempt to find its relation to the degradation of glycogen. One of us recently reported that postmortem breakdown of muscle glycogen in pike is considerably slower than that in white sucker, whereas the other biochemical changes were similar (Manohar 1970).

Glycogen phosphorylase catalyses the following initial reaction in the degradation of glycogen:

$$(\text{glucose})_n + \text{inorganic phosphate} \rightleftarrows (\text{glucose})_{n-1} + \text{glucose-1-phosphate}.$$

From this it is possible to measure the phosphorylase activity in the direction of synthesis by determination of the amount of inorganic phosphate formed from glucose-1-phosphate. It is well known that muscle phosphorylase exists in two forms: phosphorylase "*a*," which is enzymatically active, and phosphorylase "*b*," which is inactive. An enzyme, a kinase, can convert phosphorylase "*b*" to phosphorylase "*a*" in the presence of adenosine triphosphate (ATP) and Mg^{++}. On the other hand, another enzyme, a phosphatase, can convert the active form "*a*" to the inactive form "*b*" by dephosphorylation of "*a*". Also phosphorylase "*b*" becomes active in the presence of AMP (Brown and Cori 1961). Therefore, the phosphorylase activities in the muscle of sucker and pike were measured both in the absence and in the presence of AMP. Hence, the activity of the "*a*" form as well as the total activity of "*a*" and "*b*" forms was measured.

Methods and materials — White sucker and northern pike, kept well fed in an aquarium, were taken out by a hand net and killed immediately by a blow on the head. They were then beheaded, gutted, and cleaned with cold tap water. Each of five fish of each species was cut in two halves along the spinal column. Measurements of glycogen content and phosphorylase activity of dorsolateral white muscle of one side of each fish were performed on the day the fish was killed. The other side of the fish was put in ice and similar measurements

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were done after 7 days of storage. Quadruplicate samples of muscle along the length of the body were used for measurement to account for the variation from head to tail, and samples from the two halves were taken from corresponding positions.

Glycogen was isolated by potassium hydroxide digestion and precipitation with ethanol according to Good et al. (1933) and measured by the phenol–sulphuric acid method of Montgomery (1957). Muscle phosphorylase activity in the absence and the presence of added AMP was measured by the method described by Cori et al. (1955). In the actual procedure, 3 g of muscle were homogenized in 20 ml cysteine–glycerophosphate buffer, *p*H 6.0, and centrifuged. The supernatant was diluted to 100 ml with the same buffer. The buffer also contained 0.1 M NaF and 0.001 M EDTA for inhibiting the activities of phosphorylase phosphatase and dephosphophosphorylase kinase, respectively (Rall and Sutherland 1962). A preliminary incubation of 0.6 ml of diluted supernatant with 0.3 ml of 4% glycogen solution (aqueous) was carried out for 20 min at 30 C and then 0.3 ml of 0.032 M glucose-1-phosphate (prepared in cysteine–glycerophosphate buffer) either with or without AMP (0.004 M) was added and the mixture incubated further. In the preliminary tests, the release of inorganic phosphate from glucose-1-phosphate by phosphorylase was constant for at least 10 min. Incubation time of 5 min was therefore chosen for measuring phosphorylase activities. Aliquots (0.4 ml) of the incubation mixture were removed at 0 and 5 min and added to 0.4 ml of 5% trichloroacetic acid to stop the reaction and precipitate proteins. Orthophosphate in the protein-free filtrate was measured by the method of Fiske and Subbarow (1925).

Results and discussion — The phosphorylase activity, without added AMP, in sucker increased slightly in 7 days, whereas that in pike showed a significant decrease in the same period. The mean values for glycogen content (mg/100 g) and phosphorylase activity (μg P released/g) in the muscle of the two species on the two occasions were:

	Glycogen	Phosphorylase	
		Without AMP	With AMP
	White sucker		
Day 0	352.5±32.5[a]	859±118	3357±426
Day 7	1.08±0.22	1327±247	3453±456
	Northern pike		
Day 0	344.8±44.1	1331±97	4577±162
Day 7	35.3±13.4	263±61	4325±340

[a]One standard error of mean.

Phosphorylase activity in the presence of added AMP remained unchanged in the sucker and pike after 7 days of ice storage. The activity of the enzyme in the presence of AMP was remarkably higher than that in the absence of AMP, showing that the major part of the phosphorylase in the muscle was in the inactive "*b*" form. As in the previous study (Manohar 1970), in 7 days the breakdown of muscle glycogen in sucker was almost complete, whereas in pike a considerable amount of glycogen was still present.

The above results suggest that the incomplete degradation of glycogen in pike muscle may be due to a decreasing amount of phosphorylase "*a*" in the muscle. As the phosphorylase activity in the presence of AMP did not show any decrease in 7 days, in either species, it is obvious that the total amount of phosphorylase (i.e., phosphorylase "*a*" + phosphorylase "*b*") remained unchanged during storage for 7 days.

Nagayama (1961) found that the phosphorylase activity of the dorsal muscle of sea bass during storage at 0 C was reduced after 3 days to about one-half of the original activity. In these investigations only phosphorylase "*a*" activity was measured.

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Some Relations Between Zooplankton and Bunker C Oil in Chedabucto Bay Following the Wreck of the Tanker *Arrow*[1]

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CONOVER, R. J. 1971. Some relations between zooplankton and bunker C oil in Chedabucto Bay following the wreck of the tanker *Arrow*. J. Fish. Res. Bd. Canada 28: 1327–1330.

Zooplankton ingested small particles of oil that were found dispersed throughout the water column in Chedabucto Bay following the wreck of the tanker *Arrow*. As much as 10% of the bunker C in the water column was associated with zooplankton and their feces contained up to 7% bunker C. The oil had no apparent effect on the organisms. In addition to the particulate oil which was exported from the bay by hydrodynamic processes, perhaps 20% more was sedimented to the bottom as zooplankton feces.

CONOVER, R. J. 1971. Some relations between zooplankton and bunker C oil in Chedabucto Bay following the wreck of the tanker *Arrow*. J. Fish. Res. Bd. Canada 28: 1327–1330.

A la suite du naufrage du pétrolier *Arrow*, le zooplancton a absorbé de petites particules d'huile dispersées à toutes profondeurs dans les eaux de la baie de Chedabucto. Jusqu'à 10% de l'huile présente dans la colonne d'eau était associée au zooplancton dont les fèces contenaient jusqu'à 7% d'huile lourde de type C. L'huile n'avait aucun effet apparent sur les organismes. En plus de l'huile particulaire entraînée à partir de la baie suivant la dynamique des eaux, des quantités additionelles, pouvant atteindre 20%, se sont déposées sur le fond sous forme de fèces de zooplancton.

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FOLLOWING the wreck of the tanker *Arrow* in Chedabucto Bay, Nova Scotia, on February 4, 1970, particles of oil up to 1 mm in diameter were found mixed down to at least 80 m and traces of the suspended oil were followed in a band of water about 10–25 km wide extending about 180 km southwest along the Nova Scotia coast (Forrester 1971). The density of bunker C is about 0.97 or only about 5% less than that of sea water. Presumably, erosion of oil deposits on the beach and destruction of surface slicks by wave action introduced new particles into the water column at a more or less continuous rate.[2] Strong mixing under nearly isothermal conditions distributed the relatively dense oil particles throughout the upper layers. Forrester (1971) estimated that the rate of production of particles less than 1 mm in diameter, based on rates of transport from the Bay, was about 6 $m^3 \cdot day^{-1}$ during the first 2 weeks after the wreck, declining to one or so later. Forrester also found that particles between 0.01 and 0.1 mm in diameter were more or less uniformly distributed vertically while the concentration of larger particles decreased with depth.

The size range of oil particles found in Chedabucto Bay includes that of most of the natural food of the zooplankton. Therefore, it seemed desirable to see whether these oil particles were ingested by the zooplankton, and, if so, what effect they might have on the animals and conversely what effect passage through the gut of a zooplankton animal might have on the oil particles.

Methods — Zooplankton were captured in Clarke-Bumpus samplers fitted with bolting cloth having 233, 125 or 76 μ apertures, with an Icelandic high speed plankton sampler, or with conventional ½-m diameter plankton nets of the same or similar mesh sizes. Most samples were examined visually for the presence of oil particles (see Forrester 1971) and then frozen. However,

[1]BI Contribution No. 256.

[2]For a more complete report of the *Arrow* disaster and its immediate effects see "Report of the task force — operation oil" Vol. 2, 103 p. Information Canada, Ottawa, 1970.

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on several occasions, samples of zooplankton were added to a volume of sea water, previously filtered to remove any particulate matter, and allowed to void. Fecal pellets were collected from the bottom of the vessels, usually concentrated by settling and filtration and finally dried at 70 C. Other pellets were examined visually under a microscope. Examination of living animals was made with a dissecting microscope.

Chemical analyses of the feces were performed directly after hexane extraction. Some frozen zooplankton samples were extracted with hexane, after a preliminary hexane rinse to remove any oil adhering to the surface, and then the extract was purified with thin-layer chromatography. Other zooplankton–oil mixtures were allowed to separate naturally, the oil floating to the surface while the plankton settled to the bottom, after which both the water and plankton fractions were extracted with hexane. The amount of bunker C was ultimately determined by either fluorescence or spectrophotometry (Zitko 1971).

Visual observations — The dominant copepod in the plankton tows from the bay was usually *Temora longicornis*, which is rather opaque and forms a dark food bolus in the foregut region during feeding (Fig. 1*a*). Consequently, it was never certain from visual observation whether the dark material in the guts, as seen under the microscope, contained oil particles. However, on several occasions this species was observed with small, dark particles, almost certainly oil, caught in the interstices of the feeding appendages (Fig. 1*b*).

The feces of *T. longicornis* are also very compact and dark in color (Fig. 1*c*). However, when squashed on a microscopic slide, a number of pellets were seen to contain a viscous liquid with a refractive index quite different from that of water and a distinctly brownish coloration under reflected illumination. The droplets were even more obvious under phase contrast (Fig. 1*d*). Samples of bunker C from the bay had a similar appearance when finely dispersed, but natural oils produced by plants and animals in the sea were either colorless and transparent or slightly orange or reddish under the microscope.

Foreguts from *Temora* were also removed by dissection and squashed on a microscope slide. These also frequently showed droplets of a brownish oil-like liquid.

A few *Calanus finmarchicus* from the bay were observed with conspicuous brownish particles in their intestines which resembled particles of bunker C in the surrounding environment. This species is more transparent than *Temora* and the food mass and feces are not usually so compact so that it was possible to observe the passage of the particles through the animal. These particles moved back and forth in the foregut region with the food mass for some minutes and then passed into the hind gut where they were formed, along with other undigested food remains, into a fecal pellet.

Animals containing smaller "oil" particles always voided them within 24 hr and showed no signs of distress. However, Mr M. A. Paranjape (personal communication) observed for some hours several *Calanus* containing rather large "oil" particles in the foregut which they seemed unable to pass into the posterior region of the gut. Haq (1967) found that certain *Metridia longa*, which seemed to be feeding well, became moribund and died even though their foreguts were full of food. On microscopic examination he found some bit of foreign material, such as a fragment of lint or bit of wood, obstructing the passage between fore and hindgut. Probably a large oil particle might cause such a blockage with fatal consequences for the individual copepod, but there was no other evidence that bunker C was harmful to the plankton animals.

Chemical analyses — On February 28 and March 1 feces were collected from living zooplankton taken on Bedford Institute Cruise 70–007 Phase II, CSS *Dawson*, from stations 36, 37, 39 (Fig. 2). In all, six samples were collected ranging in dry weight from 14 to 119 μg. Five of the six samples showed at least traces of oil with the highest amount detected being 7% from station 37 and the next highest 2.4% from station 36. Both stations contained between 0.01 and 0.02 ppm of bunker C suspended in the water, which was near the maximum concentration observed by Forrester (1971).

Quantitative tows taken from the bay and adjacent waters (Fig. 2) between February 25 and March 5, 1970, yielded an average concentration of 34.9 ± 5.3 $\mu g \cdot m^{-3}$ bunker C in zooplankton from 5 m depth. The same 125-μ aperture netting retained, on the average, an additional 347 μg of oil, either freely suspended or adhering to the external surfaces of the plankton. In each case the variability between samples was large, the range of oil in the plankton being 0–110 $\mu g \cdot m^{-3}$ and that in particles larger than 0.1 mm being 6–1700 $\mu g \cdot m^{-3}$.[2] When the quantity of oil in the plankton

[2]My estimates of oil concentration are considerably lower than those found by Forrester (1971). In some measure this discrepancy may be due to real differences between the stations sampled; but of 18 samples which were analysed by both of us, Forrester found significantly higher concentrations in 16. It is not possible to completely resolve the source of the discrepancy at this time but Forrester's method, which involved removing a portion of the oil as it floated to the surface of a freshly caught Clarke-Bumpus tow and then estimating visually the fraction removed, is highly subjective. On the other hand, my samples were frozen before

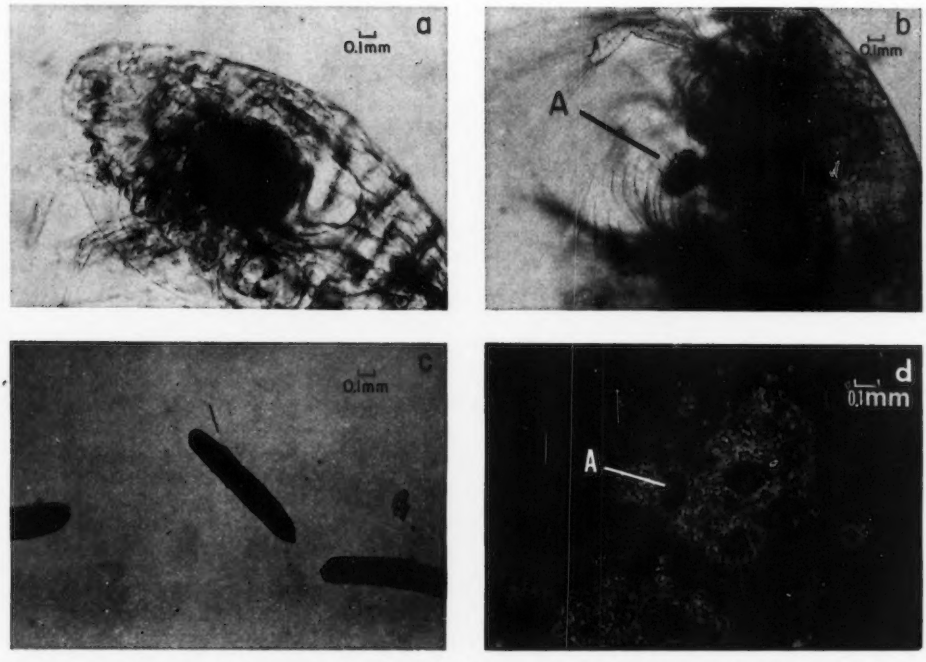

FIG. 1. *a* A living *Temora longicornis* showing dark food mass in foregut and newly formed fecal pellet in the hindgut. Indirect lighting. *b* A living *Temora longicornis* with "oil" particle (A) held by the gnathobases of the first maxillae as though about to be ingested. Indirect lighting. *c* Typical *Temora longicornis* fecal pellets. Indirect lighting. *d Temora longicornis* fecal pellet crushed under a cover slip showing "oil" droplets (A). Phase contrast lighting.

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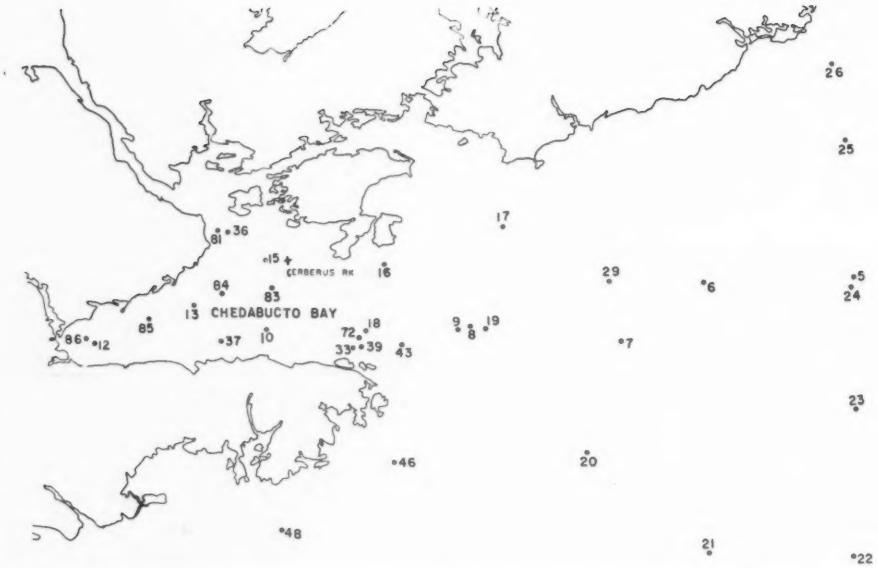

FIG. 2. Locations of plankton stations from which materials used for this report were collected.

was compared with the quantity in the water at the same station and depth, no statistical correlation could be found. Perhaps this is not too surprising because the oil particles captured by the net are almost certainly too large to be ingested by most of the animals caught in the same net.

At seven stations samples were collected from more than one depth. The average concentration of oil in the 10 samples of zooplankton analysed from depths greater than 5 m was 46.2±7.2 $\mu g \cdot m^{-3}$ which was not significantly greater than that found in the shallower layer (Mann-Whitney U-test).

Discussion — The series of samples on which this report is based suggest that, on the average, at least 400 µg of bunker C were contained in particulate form, either in zooplankton or as suspended particles, in each cubic meter of the water of Chedabucto Bay. As most of these samples were taken between February 25 and March 5, 1970, more than 3 weeks after the original disaster, it is very likely that considerably greater quantities of oil had been suspended in the same water earlier. We also know that perhaps 2% of the feces of zooplankton from the Chedabucto Bay area consisted of bunker C and that, on the average, about 10% of the suspended bunker C was directly associated with zooplankton.

If zooplankton ingested suspended oil in proportion to its concentration, in a suitable size range, in the water and if this oil passed through the animals largely unaltered to be defecated along with other undigested food remains in the form of fecal pellets which would be considerably denser than sea water, zooplankton grazing might constitute an important natural clean-up process for disposing of oil on and in the sea. What is more, fecal pellets contain a concentrated bacterial flora which might hasten the chemical degradation of the oil.

Regrettably, we have no information on the natural concentration of organic matter suspended in Chedabucto Bay prior to the *Arrow* crisis, but in St. Margaret's Bay, a similar environment just south of Halifax, we would expect to find between 100 and 300 mg organic carbon/m^{-3} suspended in the upper 5–10 m at this season (Platt and Irwin 1968). If we take these numbers as representative of Chedabucto Bay as well, and if we assume bunker C is about 50% carbon, the contribution to suspended organic matter made by the contaminating oil was between 0.07 and 0.2% using my data and up to 0.5% based on that of Forrester (1971).

Conover (1966) has shown that zooplankton on the average assimilate about 65% of their natural food supply. Assuming that bunker C is not assimilated by the animals and that it occurs as 2% of the organic matter in zooplankton feces, it can be shown by rearranging Conover's equation 9 that the concentration of oil in the ingested food

$$O_f = 1 - F_0' = \left\{1 - \left(\frac{E_0'}{1-(1-E_0')\ (U')}\right)\right\}$$

$$= \left\{1 - \left(\frac{0.98}{1-(0.02)\ (0.65)}\right)\right\}$$

$$= 0.007$$

or about 0.7%, if E_0 is taken as the non-oil fraction in the feces, F_0' as the non-oil fraction in the food, and U' as the percentage assimilated.

There are several possible explanations for the apparently higher concentration of oil in the ingested food. It is possible that my estimate of oil concentration is actually considerably too low, because I had no direct measurement of the standing stock in particles less than 0.1 mm in average dimension. Forrester measured small particles visually collected on filter paper and estimated that about 340 $\mu g/m^{-3}$ of oil occurred in this size range on the average. The amount of natural organic matter might also have been less than that found in St. Margaret's Bay at this season. The amount of oil in feces could have been considerably lower on the average or the assimilation rate lower than 65%. It is even possible that zooplankton could have selected oil for some reason. Nonetheless, the fact that oil was ingested can hardly be disputed, and for lack of a better estimate we will take 0.7% as the concentration of oil in the food of zooplankton.

To carry the argument further, we must assume some rate of feeding for the Chedabucto Bay plankton. Parsons et al. (1969) and others have shown that zooplankton can consume 15% or even more of their own organic weight in particulate organic matter each day. Only a series of samples from 19 Clarke-Bumpus plankton tows taken in Chedabucto Bay between February 12 and 16, 1970, which were preserved in formaldehyde because they contained no obvious contamination from oil, were available to provide an estimate of zooplankton biomass during the period of the *Arrow*

analysis and the separation of water, plankton, and oil was much more difficult than in the fresh samples. Loss of oil to the walls of the storage container (plastic bag) was also a probability. At present it seems wisest to look at Forrester's and my own values as upper and lower limits respectively for the true concentration of particulate bunker C in Chedabucto Bay.

crisis. These samples, taken at depths from 5 to 60 m, had an average wet weight of about 0.05 $g \cdot m^{-3}$. If we take 60 m as a reasonable average depth for the Bay and 670×10^6 m^2 as a rough estimate of its area, then the standing crop of zooplankton should be around $0.05 \times 60 \times 670 \times 10^6 = 2.01 \times 10^9$ g. Therefore, about $2 \times 10^9 \times 0.15 \times 0.10 = 3 \times 10^7$ g of organic matter are grazed, assuming organic matter constitutes about 10% of the wet weight of zooplankton. Now, if our indirect estimate of the oil concentration available to the zooplankton is about right, then $3 \times 10^7 \times 0.007 = 2.1 \times 10^5$ g or 0.21 metric tons of oil could be sedimented to the bottom of Chedabucto Bay in zooplankton feces daily.

Forrester (1971) estimated that probably not more than 100 metric tons of bunker C in particles less than 1 mm^3 was actually suspended in the bay and adjacent waters at a given moment. However, much greater quantities came ashore on beaches and headlands, which then gradually disappeared, presumably being refloated and resuspended by wave and tide. Hence, we are concerned with a dynamic situation; the important point is not how large the standing crop of suspended oil was, but rather its rate of replacement from the wreck and shoreline and, of course, its ultimate fate.

By the time our observations were made, the rate of formation of particles less than 1 mm would need to be about 1 m^3 (one metric ton) per day to account for losses caused by physical processes such as flushing (Forrester 1971). My data suggest that an additional 20% could be sedimented by zooplankton grazing within the bay itself. More would be ingested and subsequently deposited in the sediments as the particles were transported away from the Bay and gradually dispersed. If zooplankton grazing can control the natural production of organic matter, as is widely believed, very probably it will be equally effective in removing particulate organic matter artificially introduced. Indeed, the zooplankton may be the single most important natural agent leading to eventual dispersal and degradation of oil spills, at least in the open sea.

Acknowledgments — The author wishes to thank Mr M. A. Paranjape, and Dr W. D. Forrester and other members of the Bedford Institute staff who helped to collect the samples. Dr Forrester also made his original data available to the author. Dr Vlado Zitko, Fisheries Research Board, St. Andrews, and Dr Otto Hutzinger, National Research Council, Halifax, kindly performed chemical analyses for bunker C on zooplankton samples. Photomicrographs were taken with the generous cooperation of Dr Trevor Platt.

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The Zoogeographic Problem Involved in the Presence of the Glacial Relict *Pontoporeia affinis* (Crustacea Amphipoda) in Lake Washington, USA

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SEGERSTRÅLE, S. G. 1971. The zoogeographic problem involved in the presence of the glacial relict *Pontoporeia affinis* (Crustacea Amphipoda) in Lake Washington, USA. J. Fish. Res. Bd. Canada 28: 1331–1334.

The discovery in 1939 of the glacial relict amphipod *Pontoporeia affinis* Lindström in Lake Washington raised the question of the provenience of this population of the species, quite isolated as it is from the main area of relict localities of continental North America, situated east of the Continental Divide. Recent geological work and studies on the dispersal routes of freshwater fish in glacial times suggest that the *P. affinis* of Lake Washington has been able to cross the divide from proglacial waters east of it.

SEGERSTRÅLE, S. G. 1971. The zoogeographic problem involved in the presence of the glacial relict *Pontoporeia affinis* (Crustacea Amphipoda) in Lake Washington, USA. J. Fish. Res. Bd. Canada 28: 1331–1334.

La découverte en 1939 du reliquat de population glaciaire de l'amphipode *Pontoporeia affinis* Lindström dans le lac Washington soulève le problème de l'origine de cette population, isolée qu'elle est de l'aire principale de répartition, à l'est de la ligne de partage des eaux, des autres reliquats de cette espèce sur le continent nord-américain. De récents travaux géologiques et études des voies de dispersion des poissons dulcaquicoles aux époques glaciaires nous portent à croire que *P. affinis* du lac Washington, quittant les nappes d'eau préglaciaires de l'est, a pu traverser la ligne de partage des eaux.

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As is well known, numerous lakes of continental North America have proved to harbor animals belonging to the group of so-called glacial relicts (Ricker 1959; for lakes of the Canadian Islands, see Johnson 1962, 1964). Especially widespread are the crustaceans *Pontoporeia affinis* and *Mysis relicta*. Lake Washington (Washington, USA), which is inhabited by the former species, holds a unique position in that it is situated *west* of the Cordilleran Ranges; and the discovery of *Pontoporeia* there in 1939 by Scheffer and Robinson raised the question as to how it was able to reach the lake, in view of its isolation from the area of relict localities east of the Continental Divide. As has been indicated by Ricker (Segerstråle 1962), the presence in the latter region of *Pontoporeia* is readily understandable on the basis of the theory of sluicing-up of animals from northern coastal waters in proglacial lakes.

The problem under discussion was touched upon by Ricker (1959), who offers the following explanation (p. 886): "Were it not that Derzhavin (1923) reported the species from various lakes and rivers of Kamchatka, some wholly fortuitous type of dispersal for this lake [Washington] might perhaps be considered. As it is, it must be accepted that in glacial times or during deglaciation *Pontoporeia* extended far down the Pacific coast in the colder and possibly more brackish water of those times, and has since probably disappeared from the sea because of higher temperatures and salinities." He suggests that the special topography of the Vancouver region, in combination with processes of ice-flow, may have resulted in the dispersal of *P. affinis* from the adjacent sea to Lake Washington.

McPhail (1967) discusses the distribution of freshwater fishes in western Washington as related to the glacial history of the region. According to him, the events of the last (Wisconsin) glaciation, strictly speaking the Vashon Stade of the Puget glacial lobe, may be correlated with fish distribution. Geologists have concluded that the lobe advanced into the Puget Sound lowland about 15,000 years ago. The maximum penetration of the ice, the resulting drainage changes, and the pattern of deglaciation are illustrated by McPhail in a series of maps.

Concerning the prehistory of the population of *P. affinis* living in Lake Washington, McPhail reckons, as does Ricker, with invasion along a

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marine route: "When [during deglaciation] Puget Sound was first formed the sea submerged several areas that are now not part of Puget Sound. One such area was Lake Washington, which now contains two relict marine invertebrates (*Neomysis* and *Pontoporeia*) and a land-locked population of the normally anadromous smelt, *Spirinchus thaleichthys*."

In a paper on migration and prehistory of the glacial relicts of Eurasia and North America, I considered it unlikely that *P. affinis*, a lacustrine–brackish water species not tolerant of higher salinities (15–18‰ at most in the Baltic Sea) and belonging to the benthic fauna, could have migrated so far southwards from the Arctic Sea, even in glacial times. "Rather it may be expected that future geological research will provide evidence of the overflow, across the Cordilleran Ranges, along systems of valleys, of water from ancient proglacial lakes east of the ranges and thus of the existence of a more favourable path for the immigration of *Pontoporeia* to the region of L. Washington ...." (Segerstråle 1962).

The data presented in McPhail (1967) and McPhail and Lindsey (1970) seem to give the expected support to my interpretation. The following reconstruction of the prehistory of the *Pontoporeia* of Lake Washington is suggested:

Together with other faunal elements of Hudson Bay, *P. affinis* was sluiced up in front of the advancing ice-cap, finally reaching those proglacial waters that functioned as refuges and subsequently, during deglaciation, as centers for northward spread of many of the fishes considered by McPhail and Lindsey (for the sluicing-up process, see Ricker 1959). One of these ice-dammed lakes is concluded to have comprised the upper Missouri system (Fig. 1). Within its limits lie the Waterton lakes, which today harbor *P. affinis* and *Mysis relicta* (Ricker 1959). From the Missouri refuge or via other proglacial waters[1] the amphipod under discussion reached the Fraser River system and along it the area west of the Continental Divide (Fig. 1). Its transportation (*Pontoporeia* is not capable of active migration) towards Lake Washington may have taken place in ice-dammed waters in front of the Vashon Stade of the Puget glacial lobe or, alternatively, at the stage of its waning, along proglacial waters between the lobe and the continental slope. In either of these ways the amphipod reached the Puget Sound region, which during deglaciation was occupied by ice-dammed lakes (one of them was Lake Russell; McPhail 1967 fig. 10C). When the margin of the Vashon glacial lobe retreated farther north and the sea entered through the Juan de Fuca Strait, Puget Sound was formed; as was mentioned earlier, areas adjacent to the present Puget Sound, amongst them Lake Washington, are also assumed to have been submerged by that time. *Pontoporeia* may have survived that phase in nearby brackish or fresh waters, reaching Lake Washington when, after freshening of the marginal parts of the submerged area in connection with land-uplift, the lake became isolated. This interpretation is supported by the presence, in numerous fresh waters adjacent to the Puget Sound of our day, of genuine freshwater fishes (McPhail 1967 fig. 6).

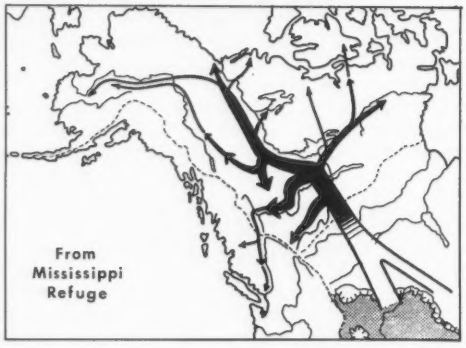

FIG. 1. The dispersal routes of freshwater fishes from the Missouri refuge (part of Mississippi refuge sensu latiore) of the last glaciation. Refuge stippled. (From McPhail and Lindsey 1970.)

The above interpretation of the prehistory of the *Pontoporeia* population living in Lake Washington should be supplemented by the following comments.

McPhail and Lindsey (1970 p. 25) reckon with postglacial exchange of freshwater fishes across the Continental Divide in both directions. Westward migration is, for instance, suggested for the brassy minnow (*Hybognathus hankinsoni*), which "successfully completed the 400-mile trip downstream through the inimicable Fraser Canyon to become established in suitable waters of the lower Fraser Valley" (p. 269). The fact that, in contrast to *Pontoporeia*, the fish is not found south of the mouth of the Fraser River is explainable by its inability to tolerate brackish water. In this connection the prehistory of the peamouth (*Mylocheilus caurinus*) deserves a note. McPhail and Lindsey

[1]McPhail and Lindsey (1970 p. 13–14) say: "In fact the Laurentide and Cordilleran ice sheets never completely met in some areas of western Alberta; isolated and perhaps shifting refuges may have persisted along the east slope of the Canadian Rocky Mountains."

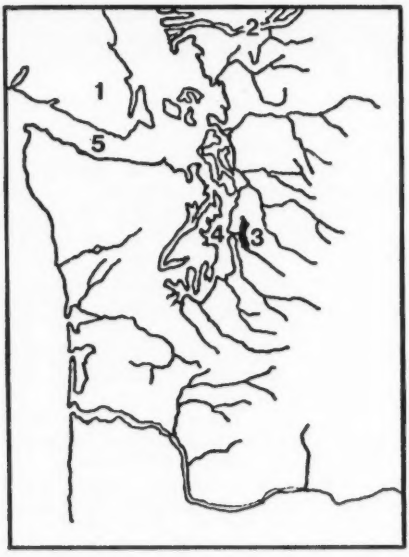

FIG. 2. Map of western Washington: 1 Vancouver Island; 2 Fraser River; 3 Lake Washington (black); 4 Puget Sound; 5 Juan de Fuca Strait.

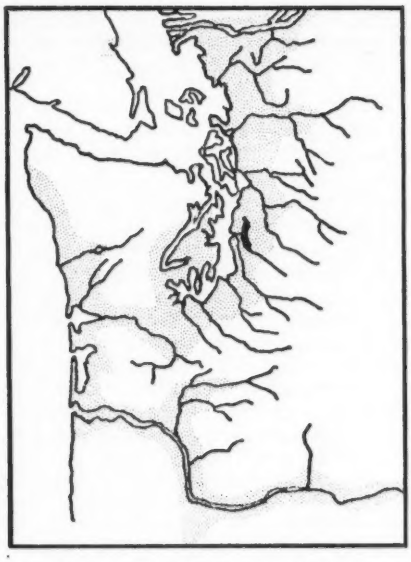

FIG. 3. Western Washington distribution of the peamouth, *Mylocheilus caurinus*. (After McPhail 1967.)

(p. 234) assume that this fish originates from the so-called Pacific refuge (see below) and that, in postglacial times, it spread north from the Columbia system into the Fraser River and from the Fraser system east across the Continental Divide into the present localities in the upper Peace and Athabasca rivers. In view of the brackish water tolerance of the peamouth (McPhail 1967 p. 4) and its distribution pattern in western Washington (Fig. 3), migration in the opposite direction seems to be a possible alternative, involving a parallel to the case of *Pontoporeia*.

The presence in Lake Washington of *Neomysis* and *Spirinchus thaleichthys*, which no doubt have migrated along a marine route in connection with the submersion of the Puget Sound area, does not affect the view presented here with regard to the prehistory of the *Pontoporeia* of the lake. Coexistence in isolated basins of faunal elements of different prehistory is no unknown feature. One may, for instance, think of the Caspian Sea, where freshwater, brackish water, and euryhaline marine animals live side by side since the isolation of the basin concerned.

As was already mentioned, it has been suggested that the Vashon Stade of the Puget Sound glacial lobe advanced into the Puget Sound lowland about 15,000 years ago. A prerequisite for the interpretation put forward in the present article is that *Pontoporeia* reached the coast at about the same time or even somewhat earlier (for the case that its transportation southwards from the mouth of Fraser River occurred in front of the ice lobe itself, see above). In fact, early deglaciation of the Fraser system seems not unlikely. It has been assumed that the ice retreated from the Seattle area before 14,000 BP (Crandell et al. 1958). Phases of temporary retreat and subsequent readvance of the Puget Sound ice lobe may also be taken into consideration. On the whole, little seems to be known about the deglaciation of the Cordilleran ice sheet. "Apparently most of the coastal area became ice-free relatively early" (McPhail and Lindsey 1970 p. 14).

It seems not unlikely that the above interpretation of the prehistory of the *Pontoporeia* of Lake Washington may contribute to the elucidation of the deglaciation of the Cordilleran ice sheet as related to the formation of the Puget Sound glacial lobe. A case in which biological work has been of help to geologists may be referred to; it is concerned with the freshwater amphipod *Gammarus pulex* (L.) and its migration into Sweden from southern continental areas during the melting phase of the last glaciation. Studies on the present distribution of the species in southern Sweden (Segerstråle 1954) have, on the one hand, modified geological

concepts of the deglaciation of the southern Baltic basin and, on the other, confirmed the sequence of ice-lakes in nearby Swedish areas (Nilsson 1958).

McPhail and Lindsey (1970 p. 11 and fig. 2) reckon with a refuge for many freshwater fishes south of the ice and west of the Rocky Mountains ("Pacific refuge"). "The Pacific slope of North America was probably isolated from the rest of the continent for a long time preceding the Pleistocene, and developed many distinctive genera and species of freshwater fishes .... The principal source from which freshwater fishes repopulated British Columbia after glacial retreat was the Columbia River basin, which probably included more lakes during the Wisconsin period than it does now."

What about the possibility that the Lake Washington population of *Pontoporeia* is derived from the Pacific refuge? One could perhaps find an argument in favor of this view in the somewhat aberrant morphology of the population concerned, on account of which subspecific status has been proposed for this form of *P. affinis* (ssp. *erythrophthalma*, Waldron 1953). However, I find such an interpretation of the prehistory of the Lake Washington *Pontoporeia* unlikely, for the following reasons: (*a*) The spread northward of freshwater fishes from the Pacific refuge is assumed to have taken place by active migration, whereas the dispersal of *Pontoporeia* is confined to passive transportation. (*b*) The morphological differences between the Lake Washington form and the normal *P. affinis* are slight, being mainly confined to color of the eyes (red against black) and the bifurcate structure of the spines of the basal lobe of the first maxilla. These divergences would not seem to be any obstacle to the view that the migration of *Pontoporeia* into Lake Washington occurred as late as the last glaciation. An argument is offered, for instance, by the rapid evolution found in another relict amphipod, *Pallasea quadrispinosa*, living in lakes of northern Eurasia. In Finland a population of this species is known that exhibits marked morphological modification, the characteristic dorsal spines being entirely missing in most specimens (Segerstråle 1958). Geological evidence allows the conclusion that the population developed this difference at most 7500 years ago, when the spring harboring it was isolated. It should also be remembered that even in the continental North American area east of the Continental Divide some morphological differentiation of *P. affinis* had been disclosed (Segerstråle 1937, 1971). (*c*) The possibilities for westward spread of aquatic organisms from the Missouri refuge suggested by McPhail and Lindsey seem to give the most natural explanation of the existence of *Pontoporeia* in Lake Washington.

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Age and Growth of the Giant Pacific Sea Scallop, *Patinopecten caurinus*, from the Strait of Georgia and Outer Washington Coast

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At any given age, giant Pacific sea scallops, *Patinopecten caurinus*, from an inner coastal area of Washington (Strait of Georgia) were about 1.5 times as large as those from an outer coastal area (near Destruction Island). Annuli were apparently formed during the winter. Lee's phenomenon, i.e., a slower estimated growth for young animals when calculated from successively older individuals than the true average growth, occurred in the outer coast data but not in the Strait of Georgia data. Differences in size between populations at the same ages apparently resulted from differences in the amount of growth during early years of life, because growth rates in later years differed little. Differences in growth rate were more likely to be due to hydrographic conditions than to infestations by boring annelids. Growth rate of the scallops in the Strait of Georgia was similar to that reported for the species off Kodiak Island, Alaska, to about 135 mm; thereafter, Strait of Georgia scallops grew more slowly.

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La pétoncle géante du Pacifique, *Patinopecten caurinus*, provenant de la zone côtière intérieure de l'Etat de Washington (détroit de Georgie) est environ 1.5 fois plus grande que la pétoncle de même âge habitant la zone côtière extérieure (près de l'île Destruction). Les annuli semblent se former en hiver. Les données de la zone extérieure réflètent le phénomène de Lee, i.e., pour les jeunes individus, une croissance estimée plus lente que la croissance moyenne réelle calculée à partir d'individus successivement plus âgés. Le phénomène n'apparaît pas dans les données du détroit de Georgie. Les différences de taille entre populations de même âge semblent résulter de différences dans la croissance des premières années, puisque par la suite les deux populations croissent au même rythme. On attribue ces différences aux conditions hydrographiques du milieu plutôt qu'à des infestations d'annélides perceurs. Jusqu'à une taille d'environ 135 mm les pétoncles du détroit de Georgie croissent au même rythme que celles de la même espèce au large de l'île de Kodiak en Alaska; passé cette longueur, les pétoncles du détroit de Georgie croissent plus lentement.

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SUCCESS of the Alaska scallop fishery (Haynes and Powell 1968) renewed interest in the scallop resource off the Washington coast. In May and June 1968, the National Marine Fisheries Service research vessel *John N. Cobb* explored for scallops from just south of Cape Flattery to the Columbia River. The survey had two major objectives: (1) to determine distribution and abundance of scallops and (2) to obtain data on their age and growth. During the survey it became apparent that the scallops caught along the outer coast were considerably smaller than those known to occur in the protected waters of the Strait of Georgia. A sample of scallops was therefore taken near Alden Bank in the Strait of Georgia to determine if the size difference between scallops from the two areas was related to rates of growth. In this report, we discuss the age and growth data, particularly in regard to the possible

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causes for differences in growth, and compare these findings with those published for sea scallops from the Gulf of Alaska.

Materials and methods — Scallops were collected along five transects at 5-fath intervals between 30 and 60 fath deep off the outer Washington coast and at a single location at 27 fath near Alden Bank (Fig. 1). The sampling gear consisted of a New Bedford-type sea scallop dredge 2.44 m (8 ft) wide with a ring bag of 76-mm (3-inch) inner diameter metal rings. This type of gear is described in detail by Posgay (1957) and Bourne (1964). About 200 of the scallops were taken for aging from catches off the outer coast and about 60 from a sample near Alden Bank. They were taken randomly from the catches; no attempt was made to keep the sexes or the scallops from individual hauls separate.

We selected height of shell instead of weight of whole animal as the basis for determining growth because increase in height is a more convenient and a more accurate measure of scallop growth. The upper (left) valves were used for the age determinations because growth rings on this valve are usually more distinct than on the lower (right) valve.

The age of scallops was based on a count of annuli (annual growth rings). Counts of annuli have been used before in growth studies of scallops, including the European sea scallop, *Pecten maximus* (Mason 1957); the North Atlantic sea scallop, *Placopecten magellanicus* (Stevenson and Dickie 1954; Merrill et al. 1966); and *Patinopecten caurinus* (Haynes and Powell 1968).

Though we were unable to tag scallops or observe a population periodically for 12 months to verify the time of formation of the annulus, we obtained data from samples from the commercial fishery in the Gulf of Alaska on six occasions during winter months and on three others (Fig. 2). We used these data in combination with back calculations of growth of the scallops from Washington to determine a first approximation of the time of annulus formation in *P. caurinus*.

The scallops in each sample from the Gulf of Alaska were examined to determine the amount of shell growth from the last annulus to the outer (growing) edge of the shell. We used only scallops with fewer than five annuli for this phase of the study because older scallops grow so slowly that their annuli are very close together. Each shell was classified as to whether an annulus was absent, was in the process of forming, or was completed at the shell edge. On December 12, 1968, 6% of the scallops from Alaska had an annulus forming at the shell margin; by January 20, 1969, 87% had completed this outer annulus (Fig. 2). A new annulus did not start forming until the following winter. Although precise dates cannot be established, it seems reasonable to conclude from the above data that *P. caurinus* form their annuli sometime during the winter months, possibly when water temperatures are lowest. Similar conclusions were reached for *Placopecten magellanicus* (Stevenson 1934; Stevenson and Dickie 1954; Merrill et al. 1966).

That spawning may have retarded shell growth enough to form annuli, as suggested for scallops

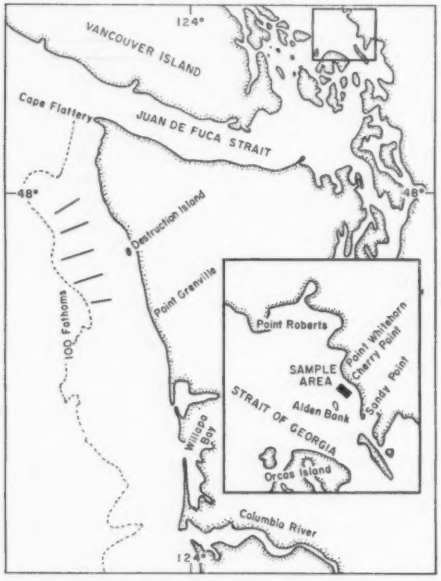

FIG. 1. Washington coast, showing locations of offshore survey transects on outer coast and of area in Strait of Georgia where sea scallops were sampled for age and growth studies.

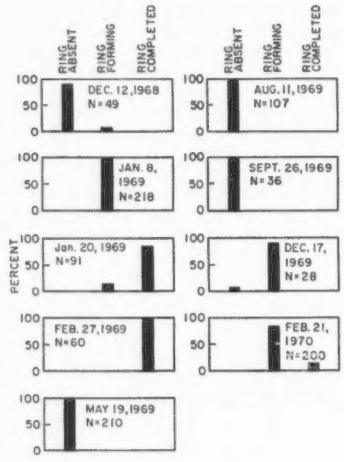

FIG. 2. Time of formation of annuli for sea scallops obtained from commercial fishery in the Gulf of Alaska.

by some investigators (Gutsell 1930; Tang 1941), was unlikely. Although information on the time of spawning of *P. caurinus* is fragmentary, it is conclusive enough to show that spawning occurs sometime during the summer and not during the winter (Hennick 1970), when it appears that the annulus is formed. In addition, spawning cannot possibly be responsible for cessation of growth during the first three winters in *P. caurinus* because they do not spawn for the first time until at least the summer after deposition of the second annulus (Haynes and Powell 1968).

Patinopecten caurinus is probably 1½ years old when the first definable annulus is formed. Although the dates of spawning and annulus formation were not known precisely in our studies, spawning seemed to occur in summer and annuli to form in winter, a time difference of about 6 months. The first definable annulus occurred at a shell height of about 18 mm in the scallops from the Strait of Georgia (Table 1) and about 9 mm in those from the outer coast (Table 2). From a consideration of the average growth for these scallops, it seems reasonable to assume that even the small amount of growth (9 mm) could not be accomplished in only 6 months (including the planktonic larval period). If this assumption is correct, then the first definable annulus in *P. caurinus* is formed during the 2nd year of life and the true age at any annulus is about one-half year more than the number of annuli. Similar results were found by Merrill for *P. magellanicus* (personal communication).

"Lee's phenomenon," defined by Ricker (1958) for fish as, "a slower estimated growth rate for young fish, when calculated from a representative sample of scales of older fish, than the true average growth at the age in question," appeared in the scallop data from the outer coast but not in the data from the Strait of Georgia (Tables 1 and 2). Why Lee's phenomenon occurred in the data from one population and not the other is unknown; both populations were sampled with the same dredge and both were unexploited commercially. Regardless of the cause, the fact remains that Lee's phenomenon did exist in the outer coast sample, and the mean growth rate shown in Table 1 is probably somewhat low.

We described growth mathematically by fitting the mean of all shell heights at each annulus above the first inflection point to the von Bertalanffy growth equation:

$$l_t = l_\infty\,[1-e^{-K(t-t_0)}]$$

where l_t is shell height at t (in years); l_∞ is the theoretical maximum shell height; K is a constant expressing rate of change in height increments with respect to t; and t_0 is the hypothetical age at zero height. The estimation of l_∞ by the usual method of the regression of shell height at age $t+1$ on height at age t may result in a poor fit of the von Bertalanffy equation (Walford 1946). We therefore used the method described by Ricker (1958) whereby the l_∞ obtained from a freehand Walford line is used as a trial value to obtain the final and more correct l_∞ by iterative computation.

The von Bertalanffy growth equations for scallops from the two sample areas are:

Strait of Georgia $l_t = 160\,[1-e^{-0.354(t-0.720)}]$
Outer coast $l_t = 106\,[1-e^{-0.436(t-1.457)}]$

TABLE 1. Mean measured heights and mean calculated heights (mm) at each annulus of sea scallops from the Strait of Georgia and the outer coast.

	Strait of Georgia			Outer coast		
Annulus	Mean measured height	Mean calculated height	Difference	Mean measured height	Mean calculated height	Difference
1	–	–	–	–	–	–
2	–	–	–	–	–	–
3	–	–	–	49.8	51.1	−1.3
4	111.9	109.9	+2.0	70.5	70.2	+0.3
5	127.4	125.0	+2.4	81.4	82.6	−0.8
6	136.8	135.4	+1.4	90.1	90.7	−0.6
7	142.5	142.6	−0.1	96.5	96.0	+0.5
8	147.1	147.8	−0.7	101.2	99.4	+1.8
9	151.1	151.5	−0.4	100.5	101.8	−0.3
10	154.4	154.1	+0.3	–	–	–
11	156.2	156.0	+0.2	–	–	–

TABLE 2. Shell height (*mm*) at each annulus for scallops from the Strait of Georgia (sexes combined) of ages III–XV.

Age	Annulus														
	1(23)[a]	2(56)	3(57)	4(31)	5(25)	6(14)	7(13)	8(10)	9(7)	10(7)	11(5)	12(3)	13(1)	14(1)	15(1)
III(1965)[b]	6.8	40.5	72.2	–	–	–	–	–	–	–	–	–	–	–	–
IV(1964)	18.0	50.0	84.7	107.8	–	–	–	–	–	–	–	–	–	–	–
V(1963)	20.0	51.2	89.7	115.0	127.2	–	–	–	–	–	–	–	–	–	–
VI(1962)	–	42.0	80.0	112.0	127.0	136.0	–	–	–	–	–	–	–	–	–
VII(1961)	18.0	43.7	80.3	107.7	125.0	134.0	140.0	–	–	–	–	–	–	–	–
VIII(1960)	19.0	45.7	82.3	111.3	126.0	137.0	141.3	145.7	–	–	–	–	–	–	–
IX(1959)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
X(1958)	18.0	49.5	84.5	110.0	126.0	136.5	143.0	147.0	151.5	155.0	–	–	–	–	–
XI(1957)	–	44.5	79.0	113.0	131.5	138.5	143.5	146.0	148.5	151.0	153.0	–	–	–	–
XII(1956)	–	43.5	81.5	112.5	129.5	139.0	146.0	150.0	154.0	157.5	159.5	161.5	–	–	–
XIII(1955)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
XIV(1954)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
XV(1953)	–	50.0	90.0	116.0	132.0	138.0	144.0	148.0	150.0	153.0	156.0	157.0	159.0	161.0	163.0
Mean shell height with 1SD	17.6±3.2[c]	44.8±5.6	79.3±8.6	111.9±5.6	127.4±5.3	136.8±5.1	142.5±5.6	147.1±3.7	151.1±4.6	154.4±4.9	156.2±3.7	160.0±3.0	159.0	161.0	163.0
Avg increase	17.6	27.2	34.5	32.6	15.5	9.4	5.7	4.6	4.0	3.3	1.8	3.8	–1.0	2.0	2.0
% increase	–	154.5	77.0	41.1	13.8	7.4	4.2	3.2	2.7	2.2	1.2	2.4	–0.6	1.3	1.2

[a]Number of measurements.
[b]Year-class.
[c]One standard deviation.

The measured heights at each age and the heights calculated from the von Bertalanffy equations (Table 1) are similar over the range of ages for which the equations were fitted. The greatest discrepancy was only 2.4 mm (at the fifth annulus of the Strait of Georgia scallops), which we considered negligible.

Age and growth — Scallops from the Strait of Georgia grew faster and to a much larger size than those from the outer coast; for any given age they were about 1.5 times as large (Table 3).

The mean percentage increases indicate that beyond the 2nd year of life the growth difference between the two populations was small. The mean annual increases in absolute terms of the two populations differed markedly during the first 4 years of life — scallops from the Strait of Georgia grew much faster than those from the outer coast. Beyond age IV the differences in annual growth were small, although the relation is less certain beyond the 8th year because of the small size of the sample. The persistence of large differences in size at the same age after age IV indicates that the differences in size of scallops in the two populations depend on the nature of growth during the early years of life; the slow growth of old age differs little whether regarded from the absolute or the relative viewpoint.

In this report we have used the average shell height, defined here by one standard deviation above and below the mean, as an index of size of scallops at a given age. On the whole, shell height is a reliable index of age to about 5 years in scallops from the Strait of Georgia and to about 4 years in those from the outer coast (Tables 2 and 3). Beyond these ages, the ranges of sizes overlap so much that a scallop of a given height might be any one of several ages. This overlap was particularly evident in the older scallops from the outer coast. The lack of overlap at the younger ages indicates that the age of scallops may be reliably identified from modes in height frequency distributions up to about 130 mm in scallops from the Strait of Georgia and about 75 mm in those from the outer coast.

Comparison of growth of Washington and Alaskan scallops — The only data comparable to ours on growth of *P. caurinus* are from scallops taken near Kodiak Island, Alaska, in January 1968 (Haynes and Powell 1968). We have modified the presentation of their data for comparison with ours (Table 4). Comparison of the mean shell heights at each annulus for scallops from the three sample areas shows that growth of *P. caurinus* from Kodiak Island was more like that of scallops from the Strait of Georgia than of scallops from the outer coast. Between ages II and V, Kodiak scallops were slightly smaller than those from the Strait of Georgia, but older Kodiak scallops were slightly larger and by age XIV were about 10% larger than those from the Strait of Georgia. Calculated asymptotic heights were 180, 160, and 106 mm for scallops from Kodiak Island, the Strait of Georgia, and outer Washington coast, respectively.

Possible effects on certain factors on growth — Several factors have been offered to explain the

TABLE 3. Shell height (mm) at each annulus for scallops from the outer coast (sexes combined) of ages III–IX.

Age	Annulus								
	1(135)[a]	2(173)	3(176)	4(162)	5(117)	6(61)	7(44)	8(8)	9(2)
III(1965)[b]	11.7	37.2	62.8	–	–	–	–	–	–
IV(1964)	9.5	28.8	56.8	81.0	–	–	–	–	–
V(1963)	7.9	24.2	45.7	69.0	84.0	–	–	–	–
VI(1962)	8.4	25.0	47.2	65.9	82.0	93.2	–	–	–
VII(1961)	8.1	23.8	45.1	63.4	78.1	89.4	97.0	–	–
VIII(1960)	7.7	24.5	45.5	63.8	78.3	88.2	96.3	103.2	–
IX(1959)	8.0	22.0	41.5	60.0	72.0	81.5	89.0	95.5	100.5
Mean shell height with 1 SD	8.6±3.1[c]	26.4±6.0	49.8±9.3	70.5±10.8	81.4±5.0	90.1±9.0	96.5±9.1	101.2±7.0	100.5
Avg increase	8.6	17.8	23.4	20.7	10.9	8.7	6.4	4.7	–0.7
% increase	–	206.9	88.6	41.6	15.5	10.7	7.1	4.9	–0.6

[a]Number of measurements.
[b]Year-class.
[c]One standard deviation.

TABLE 4. Mean measured heights of sea scallops at each annulus, 1–14, from near Kodiak Island, Alaska. Modified from Haynes and Powell (1968).

Annulus	No. measurements	Mean measured height (*mm*)
1	309	16.8
2	283	41.6
3	135	71.1
4	149	97.1
5	131	121.8
6	108	137.2
7	77	147.8
8	47	154.8
9	27	160.2
10	11	164.2
11	9	169.2
12	7	170.7
13	7	173.8
14	6	177.0

differences in growth rates of molluscs between localities and age-groups: e.g., nature of the sea bed, currents, water temperature (Fairbridge 1953; Posgay 1953; Gutsell 1930; Mason 1957). To examine the probable causes for the marked differences in growth between the scallop populations of *P. caurinus* from the Strait of Georgia and those from the outer Washington coast, we considered the effects of two factors — the presence of extensive borings in the shells and the differences in hydrographic conditions in the two sampling areas.

Shell borings — Most of the shells of scallops from the outer coast were heavily infested with a boring annelid, probably a species of the spionid polychaete *Polydora*, whereas those from the Strait of Georgia were not. This polychaete has long been recognized as being harmful to bivalves (Wells and Wells 1962; Medcof and Bourne 1964). The inner shell of scallops from the outer coast, except the smaller sizes (those less than about 80 mm or 5 years old), had been so extensively perforated and subsequently repaired that the usually smooth white surface had a dark pebbly appearance and was two or three times the normal thickness. We can only speculate on the effect of such extensive repair on the normal growth of *Patinopecten caurinus*, but it is logical to assume that diversion of so much energy to repair would result in reduced growth in height. Medcof (1949) found that the effects of extensive boring by a sponge (probably *Cliona* sp.) and subsequent shell repair in *Placopecten magellanicus* caused the bodies of badly infested scallops to be shrunken and weak; the commercial meat yield of these scallops was only about half that of uninfested scallops. He also believed that the life span of badly infested scallops was considerably shortened by the shell disease.

Why the younger scallops have little or no infestation by shell borers is not known. Medcof (1949) reasoned that perhaps the shells of young scallops are too hard or their more active swimming habits prevented the settling of the boring animals.

Serious difficulties arise in attempting to relate slow growth to shell repair in a scallop population as a whole. A comparison of infested and noninfested shells does not indicate that noninfested shells have consistently greater growth. Furthermore, the degree of repair among individual shells differs markedly but their growth rate does not. Although it is true that the population of slow-growing scallops on the outer Washington coast was heavily infested with boring organisms and the faster-growing population from the Strait of Georgia was not, the relation between infestation and growth is not conclusive. Moreover, the problem cannot be solved until the effect of individual infestation and repair on individual growth is determined.

Hydrographic conditions — The hydrographic conditions of the eastern subarctic Pacific, which includes the outer Washington coast where our samples were collected, differ markedly from those of the Strait of Georgia and in a way that might result in differences in the growth of scallops. Studies on the oceanography of the eastern subarctic Pacific have been summarized by Lane (1963), who derived a model of five seasonal stages of salinity and temperature structures, which he believed to be applicable, at least in a general way, to the entire Canadian and Alaskan coastal region. Using Lane's model, we determined that sea scallops from our outer coast sample live year-round in the oceanic halocline, a relatively stable zone of water where salinity increases rapidly from 32.6 to 33.9‰ within a short depth interval and water temperature ranges from about 6.5 to 9.0 C annually. This zone underlies a seasonally mixed layer of water, which Lane called the oceanic upper zone. It is in the upper zone that phytoplankton production occurs; the nutrients required are supplied mostly by rivers and from upwelling (Anderson 1965; Stefansson and Richards 1963). Because of the permanent halocline, the maximum depth of the upper zone is limited to about 75 m (41 fath); during periods of phytoplankton abundance, it is usually less than 50 m (27 fath).

Where we collected scallops in the Strait of Georgia, the waters are turbulent because of high tidal velocities caused by constrictions of the passages in the southern strait (Tully and Dodimead

1957; Waldichuk 1957). It is in this region of turbulence that brackish water from the Fraser River and saline water from the Strait of Georgia meet, and because of the turbulence the waters are mixed to homogeneity. The Fraser River water has high concentrations of silica, and the sea water that intrudes through the strait has high concentrations of dissolved phosphates. The mixing of these waters results in a continual renewal of plant nutrients in this area and the production of rich concentrations of phytoplankton, especially from May to October (Tully and Dodimead 1957; Légaré 1957). In addition, the strait where *P. caurinus* lives has bottom water temperatures that range from about 8 to more than 10 C (Tully and Dodimead 1957).

Thus, the Strait of Georgia and outer coast areas are different hydrographically. Scallops in the Strait of Georgia live in relatively warm turbulent waters with rich concentrations of phytoplankton, especially from May to October. On the other hand, throughout the year, scallops from the outer coast (at 50–60 fath deep) live in a zone of cold, relatively stable water considerably below the euphotic zone, and possibly, therefore, have a smaller food supply.

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Maros-Schulek Technique for Measurement of Carbon Dioxide Production in Fish and Respiratory Quotient in *Tilapia mossambica*

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KUTTY, M. N., N. V. KARUPPANNAN, M. NARAYANAN, AND M. P. MOHAMED. 1971. Maros-Schulek technique for measurement of carbon dioxide production in fish and respiratory quotient in *Tilapia mossambica*. J. Fish. Res. Bd. Canada 28: 1342–1344.

The technique used by Maros et al. (Anal. Chim. Acta 25: 390–399, 1961) for accurate measurements of total carbon dioxide in small samples has been modified for large samples of water, enabling measurement of carbon dioxide production by small aquatic animals. Data obtained from *Tilapia mossambica* by using the present technique are discussed. The method is considered to be adequate for obtaining reliable estimates of carbon dioxide production in fish.

KUTTY, M. N., N. V. KARUPPANNAN, M. NARAYANAN, AND M. P. MOHAMED. 1971. Maros-Schulek technique for measurement of carbon dioxide production in fish and respiratory quotient in *Tilapia mossambica*. J. Fish. Res. Bd. Canada 28: 1342–1344.

Nous avons adapté à de grands échantillons d'eau la méthode élaborée par Maros et al. (Anal. Chim. Acta 25: 390–399, 1961) pour la mesure précise du CO_2 total de petits échantillons. Ces modifications permettent de titrer la production de CO_2 des petits organismes aquatiques. Nous discutons les résultats de l'application de cette méthode à *Tilapia mossambica* et considérons la méthode convenable à l'estimation sûre de la production de CO_2 chez les poissons.

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THIS communication describes a modification of an existing technique (Maros et al. 1961), so far not applied to any biological system, for measuring carbon dioxide production in fish and other small aquatic animals. Data obtained on the cichlid fish *Tilapia mossambica* are also presented here. Bosworth et al. (1936) and Kutty (1968) used modifications of Van Slyke apparatus for measurement of carbon dioxide output in fish. Reviewing the earlier studies on the respiratory quotient (R.Q.) in fish, Kutty (1968) cautioned that the accuracy of the method of carbon dixoide analysis has to be considered primarily in crediting reliability to measurement of R.Q. Direct titrimetric method for measurement of carbon dioxide in water has its limitations, even though this method with some refinements can be used for measuring R.Q. in fish (Stroganov 1962; Kutty MS 1970). But the latter method also has the disadvantage that it can be used to measure only a fraction of the total carbon dioxide produced by the fish, the other portion being calculable from simultaneous measurement of the base (ammonia?) produced by the animal.

Maros et al. (1961) described a very accurate method of measurement of total carbon dioxide in small samples. The method involves isolation of carbon dioxide by distillation and subsequent titration. The sample and distillate are kept free from atmospheric contamination. The apparatus is so designed that the rate of heating and condensation can be regulated allowing the carbon dioxide isolated to be trapped directly in barium hydroxide, without any bubbling.

We have adapted the Maros-Schulek method with some modifications to enable measurement of total carbon dioxide in fairly large samples of water (up to 100 ml). Our modifications are in the following respects. In the present apparatus we normally use a sample of 50 ml, instead of 10 ml in the original unit. Accordingly

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we have enlarged the size of the receiving flask from 100 to 500 ml, and that of the distillate collecting flask from 50 to 250 ml. Volumes of solutions used have also been modified suitably. As our room temperature was around 30 C we could use hexane instead of pentane; the former is cheaper and more easily obtained locally. We used a 300-w heating mantle with a built-in regulator for heating the distillation flask.

Each determination took about 30 min as in the original Maros-Schulek unit, and this is not an improvement over the Van Slyke determination (Kutty 1968); but in some ways, such as in doing away with the mercury levelling and calculations based on pressure, temperature etc., for each determination, the present method is less cumbersome.

Reagents required — Sulphuric acid (20%), 0.01 N barium hydroxide, 0.01 N hydrochloric acid, hexane and thymol blue indicator.

Procedure — The general conduct of distillation and titration is as described by Maros et al. (1961). The procedure is described here briefly, but we shall specifically refer here to those aspects involving handling of a larger water sample for measurement of respiratory carbon dioxide.

Take 15 ml of 20% H_2SO_4 in the receiver flask (500 ml) and make it up to 250 ml with freshly boiled double distilled water. Suck up distilled water in the funnel to a level just above stopcock and close it. Connect the receiver flask to the distillation unit. Switch on and adjust the heating mantle to the required heating level. When a vigourous plume of steam has come out through the condenser, connect the distillate-collecting flask (250 ml), containing 10 ml of 0.01 N $Ba(OH)_2$ made up to 75 ml with distilled water and protected with a layer of hexane (10 ml). Now take in 50 ml of sample into the receiver flask without upsetting heating and condensation adjustment. Follow this with 25 ml of distilled water as rinse. Remove the collecting flask when the level of condensed liquid with $Ba(OH)_2$ reaches 200-ml mark in the flask. Titrate against 0.01 N HCl using thymol blue as indicator. Because the respiratory carbon dioxide is estimated from the difference in the contents of two samples taken before and after closure of the respiration chamber, a blank determination could be avoided even though it is required for obtaining the total carbon dioxide content at each level accurately. In our estimations, the distilled water used was always double-distilled and freshly boiled. All the solutions were protected by a thick layer of hexane.

Determination of carbon dioxide in fish medium — The tap water available in our laboratory could not be used directly as fish medium as its content of bound carbonates was too high for accurate measurement of carbon dioxide production. Therefore, the medium for fish was prepared by decarbonating tap water by addition of H_2SO_4 and aeration at low *p*H, which was subsequently brought up to 8.0 by adding NaOH.

The same medium could be used for about 4 days in the recirculating system used for our experiments. Water samples analysed were taken at two different times, one (series I) when the medium used was old and the other (series II) when the medium was new.

Fifty milliliter samples for carbon dioxide analyses were pipetted out of 0.5 liter of medium removed from a respirometer and kept under a thick layer of hexane in a stoppered bottle. The analyses were made using two separate units of Maros-Schulek apparatus.

The carbon dioxide values (mg/50 ml) obtained in two series of determinations are given below:

Series I

Unit I: 3.659, 3.661, 3.656
Unit II: 3.654, 3.659, 3.656

Series II

Unit I: 1.217, 1.214, 1.214
Unit II: 1.221, 1.221, 1.228

Estimations for the first series were done simultaneously in the two units, but those of the second series were done one day apart. The accuracy obtained here is quite high as in the original Maros-Schulek analyses.

Measurement of carbon dioxide production and R.Q. in Tilapia mossambica — Rates of carbon dioxide production in *T. mossambica*, estimated by the present method, along with corresponding values of oxygen consumption and R.Q.' s at various ambient oxygen concentrations, are given in Table 1. It is seen that the R.Q.'s are near unity at adequate oxygen concentrations, but the quotients tend to be higher than unity, indicating anaerobiosis, at oxygen concentrations below 2 ppm, quite similar

TABLE 1. Routine carbon dioxide output, oxygen consumption, and R.Q. in relation to ambient oxygen concentration in *Tilapia mossambica* in fresh water at 30 C. Each value represents a single determination on a separate fish. Average weight of fish is 20 g.

Mean ambient O_2 conc (*ppm*)	CO_2 output (*ml/kg per hr*)	O_2 consumption (*ml/kg per hr*)	R.Q.
5.84	239.1	260.7	0.92
5.48	116.9	117.9	0.99
3.78	217.0	227.1	0.96
3.57	186.0	177.1	0.95
2.87	160.5	211.9	0.76
2.02	110.9	54.5	2.03
1.15	85.1	21.8	3.70
0.78	78.8	21.3	3.90
0.72	74.6	23.6	3.16
0.51	67.6	9.9	6.82
0.49	72.2	15.3	4.72
0.48	124.7	6.2	19.95

to earlier observations in *T. mossambica* (Kutty MS 1970).

The data (R.Q.) obtained presently are plotted in Fig. 1 along with those obtained earlier using Stroganov's method for measuring carbon dioxide production. The distribution of the two sets of values do not suggest that R.Q.'s obtained by using a method enabling direct measurement of total carbon dioxide and those obtained from changes in dissolved carbon dioxide and ammonia concentrations, differ markedly. Thus the limited data available tend to suggest that ammonia is the only base and carbon dioxide the only acid to significantly change their contents in the medium during the respiration measurements.

Data presented in Fig. 1 suggest a high intensity of anaerobiosis in *T. mossambica* at oxygen concentrations below 2 ppm. The relation of R.Q. to ambient oxygen in goldfish, rainbow trout, and *Tilapia* is discussed in Kutty (1969 and MS 1970). Goldfish is able to sustain a R.Q. over unity (2–3) for weeks or even longer (Kutty 1968), whereas *Tilapia* is not, even though at oxygen levels about 1 ppm its average R.Q. stays at 3–8 (Fig. 1).

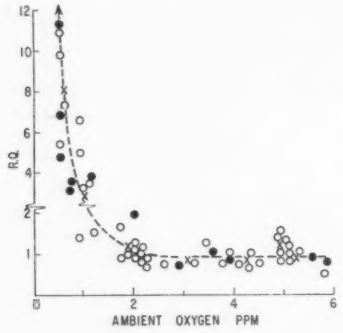

FIG. 1. Respiratory quotient in relation to ambient oxygen concentration in *Tilapia mossambica* in fresh water at 30 C. The solid points indicate the values obtained by using Maros-Schulek method for direct estimation of total carbon dioxide. The open symbols denote values obtained using Stroganov's techniques, i.e., estimating respiratory carbon dioxide from changes in free carbon dioxide and ammonia. The point with an arrow indicates a value of 20. The curve is fitted by eye through group mean values (crosses).

Information available is insufficient for a discussion of the relative merits of the different methods, but it is found that the present method is quite adequate for measurements of carbon dioxide production in fish, provided the precautions suggested earlier (Kutty 1968) are observed. The limited information available suggests that the present techniques could yield better accuracy than those previously described (Kutty MS 1966, MS 1970). From this context one may specifically point out the accuracy of the two series of determinations given here and the closer distribution of points corresponding to Maros-Schulek estimations (solid circles in Fig. 1) around R.Q. of unity, i.e., at the aerobic phase of *T. mossambica*.

Acknowledgments — We are grateful to Professor F. E. J. Fry for his interest in this work and for reading the manuscript and to M/S Arjun Singh, glass blower, and M. Muthirulappan, mechanic, Madurai University Workshop, who helped us in fabricating the apparatus.

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Anomalous Occurrence of Carp (*Cyprinus carpio*) in the Marine Environment

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BARRACLOUGH, W. E., AND D. G. ROBINSON. 1971. Anomalous occurrence of carp (*Cyprinus carpio*) in the marine environment. J. Fish. Res. Bd. Canada 28: 1345–1347.

Juvenile carp (*Cyprinus carpio*) were caught with a surface trawl in low salinity surface waters of the Strait of Georgia, British Columbia, during July 1967. A natural environmental route is established for the possible movement of carp from the Fraser River estuary to watersheds on Vancouver Island.

BARRACLOUGH, W. E., AND D. G. ROBINSON. 1971. Anomalous occurrence of carp (*Cyprinus carpio*) in the marine environment. J. Fish. Res. Bd. Canada 28: 1345–1347.

Des carpes juvéniles (*Cyprinus carpio*) furent capturées à l'aide d'un chalut de surface dans des eaux de faible salinité du détroit de Georgie, Colombie britannique, en juillet 1967. Nous traçons une route en milieu naturel pour la migration possible de la carpe depuis l'estuaire du fleuve Fraser jusqu'aux bassins hydrographiques de l'île de Vancouver.

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DURING a sampling program for larval and juvenile fish off the Fraser River plume in the southern Strait of Georgia on July 4–6, 1967, 13 juvenile carp (*Cyprinus carpio* Linnaeus) were caught with a small-mesh surface trawl towed between two vessels. The net was towed at a speed of 5.6 km/hr (3 knots) and each tow lasted 10 min. The effective mouth opening of the net was 9.6 m^2. Juvenile carp were caught at 6 stations (Robinson MS 1969) out of 24 tows made at 24 different stations (Fig. 1). All the carp were alive and active when recovered from the trawl. With these data the total estimated number of juvenile carp in the area surveyed (1550 km^2 or 450 nautical miles^2) to a depth of 2.4 m was 225,000.

McCrimmon (1968) has documented with many references the introduction and spread of carp in British Columbia and notes the apparent movement of carp within saline waters on both the east and west coasts of Canada. He noted the recent appearance of carp in the Squamish River on Howe Sound and suggested that they probably reached this watershed by moving through the brackish surface waters from the Fraser River estuary. This is substantiated by the capture of eight juvenile carp in the Strait of Georgia at three stations off the north arm of the Fraser River estuary and in other locations of the Strait of Georgia (Fig. 1). Nine carp were taken in low salinity water, about 5‰ or less; one about 3 miles off Point Grey in water of 11.5‰ salinity; one off Belle Chain Islets near Saturna Island in 13.9‰ salinity; and two were recovered from the stomach of a coho salmon

FIG. 1. Location of 24 stations (open circles) where tows were made with a two-boat surface trawl. The number beside six of the stations is the number of juvenile carp caught.

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Oncorhynchus kisutch, 142 mm fork length) in 11.5‰ salinity water off Point Grey (Table 1). For salinity and surface temperature at each of the 24 stations, see Robinson (MS 1969).

Carp are known to live and flourish in brackish water in many parts of the world. In North America the waters of the Salton Sea in southeastern California were formed in 1905 and 1906, and became more saline over the years because there was no outflow. This resulted in a depletion of the original fish stocks. However, as late as 1916 the most abundant species was the carp (Walker et al. 1961 p. 77), when salinity of the Salton Sea had reached 18.6‰ (Carpelan 1961 p. 19; Wooster et al. 1969). In Israel, carp fingerlings are grown in ponds with a salinity as high as 9‰ (Soller et al. 1965).

An isolated population of carp was at one time resident in Glen Lake on Vancouver Island (Carl et al. 1959 p. 101), but this population is believed to have been eradicated by poisoning with rotenone (McCrimmon 1968 p. 24). There are no reports at present of carp reaching the rivers of Vancouver Island, but their presence in the surface waters of the Strait of Georgia during high runoff from the Fraser River suggests that the Strait of Georgia and adjacent channels may no longer be an effective barrier. Tully and Dodimead (1957) regard the Strait of Georgia as an arm of the sea into which the Fraser River discharges, forming a brackish upper zone which moves persistently seaward entraining salt water en route. Variation in the salinity, at least in the upper zone, is primarily due to dilution of the sea water with fresh water from Fraser River drainage, and precipitation. When the Fraser River has a high discharge, particularly in June, Tabata (personal communication) has shown from aerial photographs and current measurements that some of the main discharge from the Sand Heads region (Fig. 1) takes the form of a "jet" across the Strait of Georgia toward Porlier Pass. It is quite possible for any small fish to be swept by this strong current across the Strait in about 6 hr. On an ebb tide these lower salinity surface waters are discharged through Active Pass and Porlier Pass into Trincomali Channel, at the same time sweeping juvenile fish along in the fast current.

Mr R. Noble, attendant at the lighthouse on Galiano Island at Porlier Pass, reports in his diary of daily seawater observations that cloudy green water and even muddy water flowed through Porlier Pass on some of the ebb tides in June 1967. After the lower salinity surface waters pass through Porlier Pass and Active Pass, isolated pools or "clouds" of low salinity water may form and merge with surface waters discharged from the Chemainus or Cowichan rivers. Thus a natural environmenta

TABLE 1. The number, fork length, and weight of juvenile carp caught with a two-boat surface trawl in the Strait of Georgia, with surface temperature and salinity at corresponding stations.

Date July 1967	Station no.[a]	No. carp caught	Fork length (*mm*)	Wt (*g*)	Surface temp (*C*)	Surface salinity (‰)	Location
5	11	1	40	1.35	21.3	13.9	Off Belle Chain Islets near Saturna Is.
5	16	3	26	0.43	21.5	<5.0	Off Canoe Pass Buoy, Roberts Bank, Fraser R.
			35	0.93			
			37	1.17			
6	19	4	26	0.34	21.4	<5.0	Off Sturgeon Bank, North Arm Fraser R.
			35	0.99			
			37	1.20			
			41	1.52			
6	20	1	35	0.99	21.5	5.2	Off North Arm Fraser R. by Point Grey
6	21	1	38	1.13	20.5	11.5	3 miles off Point Grey
		2[b]	30				
			35				
6	23	1	38	1.0	20.2	5.5	8 miles off Sand Heads Light, Fraser R.

[a]Robinson 1969 p. 7.
[b]Taken from stomach of coho salmon, *Oncorhynchus kisutch*.

route is established for the possible movement of carp from the Fraser River estuary to other watersheds on Vancouver Island.

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First Record of the Bluespotted Poacher, *Xeneretmus triacanthus*, in British Columbia

ALEX E. PEDEN AND C. G. GRUCHY

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PEDEN, A. E., AND C. G. GRUCHY. 1971. First record of the bluespotted poacher, *Xeneretmus triacanthus*, in British Columbia. J. Fish. Res. Bd. Canada 28: 1347–1348.

A single specimen of the bluespotted poacher (*Xeneretmus triacanthus*) provides the first record of this species from British Columbia. The specimen, which extends the known range from Puget Sound to Kwatna Inlet, is described in detail.

PEDEN, A. E., AND C. G. GRUCHY. 1971. First record of the bluespotted poacher, *Xeneretmus triacanthus*, in British Columbia. J. Fish. Res. Bd. Canada 28: 1347–1348.

Cette première mention de l'agone bleu tacheté dans les eaux de la Colombie britannique repose sur un seul spécimen que les auteurs décrivent en détail. L'aire de distribution de l'espèce se trouve, par le fait, élargie depuis le détroit de Puget jusqu'à l'anse Kwatna.

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UNTIL the present, the range of the bluespotted poacher (*Xeneretmus triacanthus*) was considered to extend from San Diego, California, to Puget Sound, Washington (Jordan and Starks 1895; Schultz 1936). On the basis of a single specimen captured in Kwatna Inlet (52°03′N, 127°35′W) the known range of this species must now be extended more than 250 miles northward into the waters of British Columbia. This specimen was taken by H. P. Arai and J. W. Scoggan in a 4.5-m (15-ft) "shrimp trawl" on May 28, 1965.

To facilitate comparison of this northerly specimen (National Museum of Natural Sciences catalogue number NMC65-258) with others, a description is provided.

Methods — Counts and measurements follow the definitions of Hubbs and Lagler (1958) except that in our specimen the last rays of the soft dorsal and anal are considered unbranched. Terminology of the bony body plates follows Gruchy (1969). Seniority of authorship follows the principle of McPhail and Lindsey (1970 p. 2).

Description — Counts. Fin rays: first dorsal, V; second dorsal, 6; anal, 6; pectorals, 13; pelvics, I, 2. Lateral line: 40 pores, 40 plates. Plates on body: total dorsals, 39 (including 23 paired dorsolaterals and 16 median dorsals); supralateral, 41 (plus 1 on caudal fin); in-

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fralateral, 38 (plus 1 on caudal fin); total ventral, 38 (including 20 paired ventrolaterals and 18 median ventrals). First dorsal spine between dorsolaterals 8 and 9; last dorsal spine between dorsolaterals 11 and 12; first dorsal ray between dorsolaterals 17 and 18; last dorsal ray between dorsolaterals 21 and 22; first anal ray between ventrolaterals 15 and 16; last anal ray between pair of ventrolaterals 20. Series of plates, between vent and anus and bounded laterally by ventrolaterals, 16; on breast, 27; on posterior branchiostegal membrane near isthmus (minute), 3; in gular region, 3. Plates beneath suborbital ridge, 3. Bony ossicles on left eyeball, 3; on right eyeball, 4.

Measurements: standard length 158.5 mm (remainder of measurements expressed as thousandths of standard length); head length 221; snout length 63; greatest orbit diameter 69; bony interorbital width 15; body depth 102; body width at pectoral fin base 121; caudal peduncle depth 19; predorsal distance 333; preanal distance 517; origin of first dorsal to origin of second dorsal 181; longest dorsal spine 74; longest dorsal ray 74; longest anal ray 88; longest pectoral ray, 192; pelvic fin length 79; caudal fin length 95.

Body elongate, slender. Head somewhat depressed; mouth terminal, small; jaws about equal; maxilla reaching a little more than halfway from snout to orbit; snout pointed. Rostral plate free, small, terminal; pair of ventrolateral rostral spines small (median upright rostral spine broken off on our specimen). Paired nasal, postocular, parietal, and occipital spines prominent; suborbital, preopercular, and suprascapular spines present but not prominent. Two round fleshy barbels at posterior end of maxilla, the first about one-half as long as the second; on the right ramus of the lower jaw, 3 barbels, on the left, 4, all of these occurring near pore openings. Teeth on dentaries, premaxillae, vomer, and palatines. Branchiostegal membranes joined to isthmus but with no free fold posteriorly. No occipital pit, but nape depressed posterior to occiput. Plates on body in all series (except median ventrals) spinous. Origin of anal directly below origin of second dorsal. Pectoral fin notched between ninth and tenth rays, and lower four rays exserted. Caudal truncate.

Colour (after preservation): brown, lighter on venter; six dark blotches laterally, the first situated at upper base of pectorals. Distal half of pectoral rays dusky, as well as upper pectoral rays near base. Dorsal spines darkly pigmented, but membrane of this fin not pigmented; rays of second dorsal and caudal dark, distal half of membrane of these fins dusky; anal fin membrane colourless, but with distal half of rays dusky.

The specimen is a male.

Discussion — All other species of Agonidae recorded from British Columbia to date have fewer than two maxillary barbels, or the origin of the anal fin is anterior to the origin of the second dorsal. *Xeneretmus triacanthus* is readily distinguished by the presence of two barbels at the tip of each maxilla and by having the origin of the anal directly below the origin of the second dorsal. In addition, *X. triacanthus* has two or three bony plates beneath the suborbital; all other known species of *Xeneretmus*, including those not recorded from Canada, have the cheek beneath the suborbital naked (Fitch 1966; Freeman MS 1951). The counts and morphometric measurements of our northerly specimen fall within the limits previously described for this species (Jordan and Evermann 1898).

The bluespotted poacher is generally reported from waters more than 40 fath deep (Freeman MS 1951; Jordan and Evermann 1898). The present specimen was captured about 90 m (100 yards) offshore, but we are uncertain of the depth at which it was taken. Other species of fishes captured along with *X. triacanthus* in this collection included: seven specimens of *Sebastes maliger*, one *Icelinus borealis*, two *Lyopsetta exilis*, one *Hippoglossoides elassodon*, six *Lepidopsetta bilineata*, one *Atheresthes stomias*. The collectors reported that the bottom was hard and that sponges and crinoids were present.

Acknowledgments — We thank Dr J. L. Hart for his review of our manuscript.

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A Notacanthid *Macdonaldia challengeri* Collected off the Oregon Coast

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STEIN, D., AND J. BUTLER. 1971. A notacanthid *Macdonaldia challengeri* collected off the Oregon coast. J. Fish. Res. Bd. Canada 28: 1349–1350.

A fifth specimen of a rare deep-sea spiny eel, *Macdonaldia challengeri*, is reported, extending the known range in the North Pacific Ocean to the Oregon coast.

STEIN, D., AND J. BUTLER. 1971. A notocanthid *Macdonaldia challengeri* collected off the Oregon coast. J. Fish. Res. Bd. Canada 28: 1349–1350.

L'auteur mentionne un cinquième spécimen d'anguille épineuse abyssale rare, *Macdonaldia challengeri*, ce qui étend à la côte d'Orégon l'aire de répartition connue de cette espèce dans le Pacifique-Nord.

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A female notacanth *Macdonaldia challengeri* Vaillant (1888), 490 mm standard length (OSU collection number 1891), was captured in a 3-m beam trawl at a depth of 2450 m, 120 km, west of Cape Falcon, Oregon (45°39.4′N, 125°57.3′W) by R/V *Yaquina* on March 19, 1970. The bottom temperature at this station was 2.03 C, salinity was 33.75‰, and oxygen was 0.829 ml/liter. The bottom sediment was mud.

Four specimens of *M. challengeri* have been previously reported: the holotype off Japan in 3429 m (Vaillant 1888); one in the Bering Sea in 2971 m (Gilbert 1896); and two off Vancouver Island in 2103–2196 m (Peden 1968).

Marshall (1962) divided the Notacanthidae into three genera according to the structure of the dorsal fin: *Notacanthus* (6–12 spines plus 1–2 rays, medium- to long-based); *Polyacanthonotus* (29–37 spines plus 1 ray, long-based); and *Macdonaldia* (27–34 spines, long-based). *Macdonaldia* is considered a synonym of *Polyacanthonotus* by S. McDowell (unpublished data).

Although morphometric characters of our specimen are close to those previously reported, several meristic characters are not within the range of *M. challengeri* previously reported by Peden (1968), who summarized the previous morphometric and meristic data in a table. Our specimen has 37 dorsal spines (previous range 32–35), 15 pectoral fin rays (previous range 11–13), and 10 pelvic fin rays (previous range 8–9). There are 17 gill rakers on the first gill arch (previous report, 14). Fin spine and ray counts are highly variable in this family, however, even between individuals captured in the same trawl (S. McDowell personal communication).

The description of the specimen generally follows that of Peden (1968), with the following elaboration: two nostrils immediately adjacent, not separated by scales, maxillary extending behind the posterior nostril. One row each of maxillary, palatine, and mandibular teeth. Articulation of jaw horizontally level with ventral end of first gill cleft. Second dorsal spine on vertical through pectoral base. Tips of short pelvic fins almost reaching anus. Intestine yellow; ovaries paired, long, thin, dorsolaterally in posterior two-thirds of body cavity. Body cavity extending short distance behind anus. Peritoneum dark brown.

Color in alcohol: gray with brown around scales; pectoral and pelvic fins brown; operculum blue, shading into black at posterior edges; interior of mouth and gill cavity black.

The stomach contained about 2 ml of material, mostly unidentifiable. All identifiable material consisted of crustacean fragments, including the anterior part of a small shrimp and the abdomen of a tanaid-like isopod. The intestine contained about 3 ml of unidentifiable material, a few crustacean fragments, one parasitic nematode, and 15 digenetic trematodes.

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Infectious Pancreatic Necrosis of Salmonids in Ontario

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SONSTEGARD, R. A., AND L. A. MCDERMOTT. 1971. Infectious pancreatic necrosis of salmonids in Ontario. J. Fish. Res. Bd. Canada 28: 1350–1351.

Infectious pancreatic necrosis (IPN) virus was isolated from brook trout (*Salvelinus fontinalis*) in two hatcheries of the Ontario provincial salmonid hatchery system. No histories of abnormal mortalities suggestive of IPN infection had been reported at any of these hatcheries.

SONSTEGARD, R. A., AND L. A. MCDERMOTT. 1971. Infectious pancreatic necrosis of salmonids in Ontario. J. Fish. Res. Bd. Canada 28: 1350–1351.

Nous avons isolé le virus de la nécrose pancréatique infectieuse (NPI) d'ombles de fontaine (*Salvelinus fontinalis*) provenant de deux établissements de pisciculture du réseau provincial de pisciculture des salmonidés. Aucun cas de mortalité anormale, suggérant une épidémie de NPI, n'avait été rapporté a l'un ou l'autre de ces établissements.

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ALTHOUGH numerous isolations of the causative agent of infectious pancreatic necrosis (IPN) have been reported in the USA and in Europe, only three definite isolations of the virus have previously been reported in Canada (Sonstegard et al. 1969; MacKelvie and Artsob 1969; Sonstegard 1970). This note reports the results of a survey (Table 1) to determine the prevalence of this disease in part of the salmonid hatchery system of the Ontario government.

A total of 2400 fish from six of these hatcheries was examined. They included 1404 brook trout (*Salvelinus fontinalis*), 707 lake trout (*Salvelinus namaycush*), 84 splake (*S. fontinalis* × *S. namaycush*) and 205 rainbow trout (*Salmo gairdneri*). Their ages ranged from 0+ to 12+ years. The methods employed in the examinations of organs and feces for the isolation and identification of IPN virus utilized RTG-2 cells and were essentially those outlined by Wolf (1968) and Wolf et al. (1968).

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The first assays for IPN virus were carried out between November 1968 and February 1969 and involved examinations of the organs of random samples of the brood stocks of brook trout, splake, and rainbow trout from five of the hatcheries (B to F). At this time, no IPN was detected. During March 1969 in similar examinations of samples of all the fish at hatchery A, IPN virus was detected in the brook trout yearlings. In April 1969 in examinations of the feces from random samples of the brood stock fish at hatcheries B, C, and D, IPN virus was detected only in the brook trout at hatchery B. These were the parent fish of the yearling brook trout in hatchery A previously found to be infected with IPN. During December 1970 an organ assay survey was made of samples of all the fish in hatchery B, and IPN was detected again in the brook trout brood stock.

In the survey, IPN was detected in regions where the disease had been unknown and unsuspected. No histories of abnormal mortalities suggestive

TABLE 1. Survey of some Ontario government salmonid hatcheries for IPN.

Date examined	Species	Age	No.	Type of assay	IPN detected
		Hatchery A			
Mar. 69	Brook trout	Fry	500	Organ	No
"	" "	Yearling	200	"	Yes
"	" "	?[a]	5	"	No
"	" "	?[b]	5	"	No
"	Lake trout	Fry	500	"	No
"	Lake trout	?[b]	5	"	No
"	Rainbow trout	?[b]	5	"	No
		Hatchery B			
Feb. 69	Brook trout	Brood stock	33	Organ	No
Apr. 69	" "	Brood stock	160	Feces	Yes
Dec. 70	" "	Fry	150	Organ	No
"	" "	Yearling	146	"	No
"	" "	Brood stock	29	"	Yes
"	Lake trout	Fry	146	"	No
"	Lake trout	Brood stock	56	"	No
"	Rainbow trout	3-year	5	"	No
		Hatchery C			
Feb. 69	Brook trout	Brood stock	16	Organ	No
Apr. 69	Brook trout	Brood stock	160	Feces	No
		Hatchery D			
Feb. 69	Rainbow trout	Brood stock	35	Organ	No
Apr. 69	Rainbow trout	Brood stock	160	Feces	No
		Hatchery E			
Dec. 68	Splake	Brood stock	33	Organ	No
		Hatchery F			
Nov. 68	Splake	Brood stock	51	Organ	No

[a]From headwaters of hatchery.
[b]Display fish in hatchery.

if IPN infection had been reported at either hatchery A or B. Although the virus apparently had little effect on the brook trout in both these hatcheries, it caused 40% mortality when inoculated into brook trout obtained from a different source.

Acknowledgments — This survey was made possible by the cooperation of and financial assistance from the Ontario Department of Lands and Forests.

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Increased Predation by the Torrent Sculpin, *Cottus rhotheus*, on Coho Salmon Fry, *Oncorhynchus kisutch*, During Moonlight Nights

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PATTEN, B. G. 1971. Increased predation by the torrent sculpin, *Cottus rhotheus*, on coho salmon fry, *Oncorhynchus kisutch*, during moonlight nights. J. Fish. Res. Bd. Canada 28: 1352–1354.

In stream aquaria, the percentages of coho salmon fry removed by the torrent sculpin averaged 70 in four tests on moonlight nights and 24 in seven tests on dark nights.

PATTEN, B. G. 1971. Increased predation by the torrent sculpin, *Cottus rhotheus*, on coho salmon fry, *Oncorhynchus kisutch*, during moonlight nights. J. Fish. Res. Bd. Canada 28: 1352–1354.

Au cours de quatre essais effectués à la clarté de la lune dans des viviers en rivière, le pourcentage moyen d'alevins de saumon (*Oncorhynchus kisutch*) coho capturés par le chabot torrenticole (*Cottus rhotheus*) a été de 70 comparativement à 24 lors de sept essais par nuits sans lune.

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IN the Pacific Northwest, sculpins (*Cottus* spp.) commonly live in the same streams as young salmon (*Oncorhynchus* spp.) and often prey on them. Field studies in the spring showed that 0–30% of the predatory-sized sculpins (over 60 mm total length) in a collection had eaten salmon; intensity of predation varied between streams and within the same stream on different dates. Preliminary studies in stream aquaria on bright moonlight nights indicated exceptionally high rates of predation and other observations suggested predation was greatest at evening and at dawn, little during dark nights, and virtually nonexistent during full sunlight. This implies sculpins may have some advantage over salmon at reduced light levels and that predation may vary with intensity of moonlight. Knowledge of this information would be helpful in planning research on predation and in planning release of artificially propagated fish.

I subjected coho salmon, *O. kisutch*, to predation by torrent sculpin, *C. rhotheus*, in stream aquaria for periods when nights were moonlit and when nights were dark to show the influence of moonlight nights on intensity of predation. Experiments were conducted during 1966 in aquaria at Cedar River near Ravensdale, King County, Washington.

Materials and methods — The facilities consisted of stream and holding aquaria that received water from the Cedar River (Fig. 1). Both types of aquaria were constructed of plywood, and interiors were painted

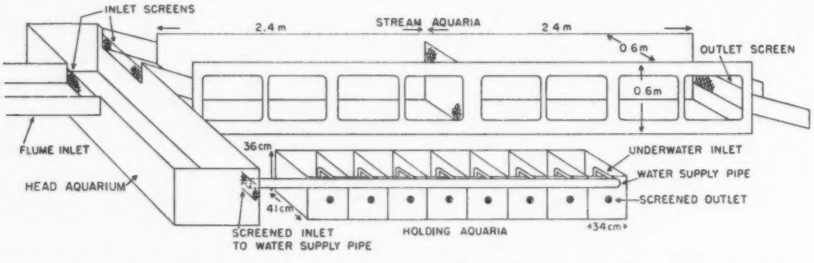

FIG. 1. Diagram of the facilities used to study predation by torrent sculpins on salmon fry.

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black with asphalt emulsion. Each aquarium had windows that faced a central aisle for observation. The windows were covered with a black plastic curtain to minimize visual disturbance to the fish, and netting over the aquaria prevented predation by birds. The aquaria were partly shaded from celestial light.

Stream aquaria for the predation tests were formed from an open trough 4.8 × 0.6 × 0.6 m high (Fig. 1). There were stoplogs and screens at both ends and at the center of this trough, dividing it into two aquaria. The stoplogs maintained water depth at 18 cm, and the screens retained the fish. The substrate in the aquaria ranged from rocks 6 cm in diameter near the window to sand at the opposite side. Water depth was 18 cm on the window side and diminished to about 2 cm over the sand. Water continously flowed through the two aquaria at an average rate of 50 liters/min.

The holding aquaria for maintaining fish before tests consisted of eight conjoined aquaria, each 41 × 34 × 36 cm high (Fig. 1). Each aquarium had a water circulation system consisting of a submerged inlet and a screened outlet that maintained the water at a depth of 18 cm. Four rocks, maximum dimension 10 cm, were placed in each aquarium. Water flow was 2.3 liters/min.

Water from the Cedar River, diverted at a low dam, was supplied by gravity flow to the head aquarium through a flume. Temperatures taken at 8 AM during the study ranged from 5.3 to 6.7 C for dark nights and from 8.1 to 9.7 C for moonlight nights (Table 1). Screens at the inlets barred debris and drift organisms. Thus, the only food available to the sculpins was the salmon.

Experimental coho salmon were collected by seining in Rock Creek of the Cedar River drainage. Torrent sculpins were collected by electrofishing in Soos Creek of the Green River drainage in King County. Total lengths of the torrent sculpins varied from 70 to 117 mm (Table 1); coho salmon had fork lengths between 35 and 42 mm.

Procedures were to collect 20 torrent sculpin and place them in the holding aquaria the 1st day of the experiment. On the 2nd day, I collected 20 coho salmon and placed 10 in each of the stream aquaria. Ten torrent sculpin were placed in each stream aquarium with coho salmon on the 3rd day.

The sculpins were maintained in the holding aquarai without food 48 hr prior to placing them with the coho salmon in stream aquaria. This was done to provide sculpins time to void food from their alimentary tract and to increase their hunger (my unpublished data indicates a 48-hr period of starvation increases the rate of torrent sculpin predation on coho salmon seven times over a 24-hr period). Thus, I assume the predatory volition of all sculpins was intensified and similar.

Experiments were conducted over 2-day periods that had either two dark nights (quarter moon to new moon) or two bright moonlit nights (within 3 days of a full moon on cloudless nights). Four experiments were made during days with bright nights and seven during days with dark nights.

TABLE 1. Percentage mortality of coho salmon from predation by torrent sculpins during 48-hr periods of dark and moonlight nights.

Date of end of test (*1966*)	Type of night and (water temp)[a]		% mortality of 10 coho salmon[b]	Length of 10 torrent sculpins (*mm*) Mean	Range
Mar. 25	Dark	(5.3)	20	89	77–105
28	"	(5.8)	20	87	70–101
Apr. 4	"	(5.8)	40	95	75–117
9	"	(6.7)	20	94	74–109
13	"	(5.6)	30	90	73–113
16	"	(6.4)	30	91	70–110
19	"	(5.6)	10	97	81–116
Avg		(5.9)	24.3		
May 3	Moonlight	(8.1)	80	93	73–112
3	"	(8.1)	50	92	73–107
6	"	(9.7)	80	85	72–109
6	"	(9.7)	70	84	74–95
Avg		(8.9)	70.0		

[a]Average water temperature (C) for 2 days of test at 8 AM.

[b]Fork lengths of coho salmon ranged from 35 to 42 mm.

Counts of coho salmon in the aquaria were made during testing and at the end of the test. Numbers of coho remained the same during daylight hours but decreased during the period from late afternoon (5 PM) to the following morning (7 AM). This observation suggests that predation probably occurred between evening and dawn.

Results — Average mortalities of coho salmon were 70% during periods with bright moonlight nights and 24% during those with dark nights (Table 1). These differences are probably a result of a visual advantage the sculpin over the coho salmon and possibly are influenced by the behavior of the coho salmon. Temperatures were higher during the moonlight experiments, but the differences in temperature within or between experimental groups was probably too small to affect results.

The visual advantage of the sculpin is suspected to result from their vantage point of vision rather than from greater acuity at lower intensities of light. Salmon positioned off the bottom would have difficulty in distinguishing the dark sculpins against a dark bottom, whereas to the sculpins the salmon would appear as dark silhouettes against a light background. The effect of the behavior

of coho salmon to maintain contact with the bottom during dark nights, probably as a means of maintaining their positions in moving water (Hoar 1953; Chapman 1962), may make them more difficult for the sculpin to see. Coho salmon were active, presumably feeding close to the surface during periods of bright moonlight (Brett and Groot (1963) determined that coho salmon feed at light intensities below that resulting from moonlight). This would provide the sculpin with a visual advantage and facilitate their capturing coho salmon over a prolonged period on moonlight nights.

Acknowledgments — I thank J. R. Heath and other personnel of the City of Seattle Water Department who granted me use of the flume site within a secured area.

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Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

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Halifax, Nova Scotia

(9) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

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